

ACUTE NEUROENDOCRINE, PULMONARY AND CARDIOVASCULAR
RESPONSES TO EXERCISE IN HEART TRANSPLANT RECIPIENTS

By

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Orthotopic heart transplantation (Tx) results in cardiac denervation. The consequences of denervation are attenuated chronotropic and inotropic reserve and the absence of an immediate tachycardic response during exercise. Recent evidence suggests that physiologic mechanisms other than cardiac denervation may be responsible for the exercise intolerance observed in heart transplant recipients (HTR). However, the available data regarding these mechanisms are sparse. The present study was designed to answer three related questions: 1) Does Tx alter the neuroendocrine response to exercise? 2) Does Tx and immunosuppression therapy adversely affect pulmonary function? and, 3) What is the skeletal muscle strength in previously deconditioned HTR placed on corticosteroid therapy? Eleven HTR, 50 ± 14 (mean $\pm$ SD) years of age, were studied prior to and 18 months after Tx and compared to 11 control subjects (CTR) matched by gender, age, height and

weight. Peak $\dot{V}O_2$ in HTR was 57% of CTR ($p \leq 0.05$) and consistent with previous research. Neuroendocrine activity, arterial blood gasses (ABG) and cardiac hemodynamics were measured during two 10 minute periods of cycle exercise at 40 and 70% of peak power output (PPO). Relative change in cardiac output (CO) was similar ($p \geq 0.05$) in HTR and CTR, but CO was augmented through increased stroke volume, not exercise tachycardia, in HTR. Plasma renin activity, norepinephrine, atrial natriuretic peptide and vasopressin responses were greater ($p \leq 0.05$) in HTR than CTR. However, an exercise intensity $>40\%$ of PPO was required to evoke the heightened response. Pulmonary function (DLCO, FVC, FEV₁) improved ($p \leq 0.05$) from pre- to postTx but all pulmonary measures in HTR were less ($p \leq 0.05$) than CTR. ABG were normal in CTR but 5 of 11 HTR became hypoxemic during submaximal exercise at 70% of PPO (15-38 mmHg below resting values). Although lean body mass was similar in both groups, knee-extension (quadriceps) strength in HTR was 69% of CTR ($p \leq 0.05$). These data demonstrate that reduced exercise capacity in HTR is the product of factors other than intrinsic cardiac performance. Abnormalities in pulmonary function and muscle strength may be the persistence of pre-existing conditions characteristic of congestive heart failure. It is probable that the exercise induced neuroendocrine overactivity was due to cardiac deafferentation, but further experiments are necessary to confirm this hypothesis.

CHAPTER 1 INTRODUCTION

Heart transplantation (Tx) is now an accepted treatment for the projected 15,000 end-stage cardiac disease patients who qualify annually (Schroeder and Hunt, 1987). At one year postsurgery, orthotopic heart transplant recipients (HTR) are reported to have fewer problems with fatigue and lack of energy than coronary artery bypass graft patients one year postbypass (Meister et al., 1986). Nonetheless, 70% of coronary bypass patients (typically older) are working within one year but only 30-35% of HTR ever return to full time employment (Meister et al., 1986).

In the past, HTR have not been considered serious candidates for career rehabilitation because of concerns regarding 1) infection and donor organ rejection, and 2) decreased functional status. Recent advances in immunosuppressant drugs, (e.g. cyclosporine, azathioprine, OKT3 and prednisone) and periodic transvenous endomyocardial biopsies have greatly reduced the incidence of organ rejection and infection with survival rates now exceeding 90% for the first 2 years and greater than 80% for the first 5 years after surgery (Heck et al., 1989). Exercise capacity, a major component of functional status, is improved after Tx but despite normal resting hemodynamics, many HTR have some degree of exercise intolerance. The explanation for the decreased exercise capacity, however, remains controversial.

Stevenson et al. (1990) recently reported that exercise capacity is low in HTR (New York Heart Association functional class 3 or 4). Numerous other studies have reported that HTR have peak systemic oxygen consumptions ($\dot{V}O_{2\max}$) that are approximately one-half to two-thirds of normal predicted values (Pope et al., 1980; Savin et al., 1980; Sietsema et al., 1987; Banner et al., 1988; Kavanagh et al., 1988; Meyer et al., 1989; Quigg et al., 1989). On the other hand, comparably good exercise capacity has been observed in some HTR. McLaughlin and associates (1980) and Yusuf et al. (1985) showed similar exercise capacity in normal individuals and HTR. Additionally, Kavanagh and coworkers (1988) have demonstrated that chronic exercise training can increase maximal exercise capacity, with the most compliant HTR approaching normal values for $\dot{V}O_{2\max}$ after 16 months of endurance exercise training. Finally, individual HTR have competed in triathlon events, the Boston Marathon and performed successfully as collegiate and professional athletes (Kavanagh et al., 1986; Golding and Mangus, 1989; Thompson, 1990). Thus, it cannot be stated with certainty at present whether a limitation of exercise capacity is inherent with Tx per se, or if other factors are responsible.

Efferent cardiac denervation, a necessary consequence of Tx, alters the heart rate response to dynamic exercise. Numerous animal and human studies have shown that the denervated heart is characterized by a high resting rate, a diminished chronotropic reserve during exercise and a slow linear decline in heart rate following exercise (Schroeder, 1979; Savin et al., 1980; Pope et al., 1980; Pflugfelder et al., 1987; Nixon et al., 1989; Colucci et al., 1989; Quigg et al., 1989). Consequently, HTR show reduced maximal cardiac output and altered cardiac output kinetics during the rest to

exercise transition (Banner et al., 1988). Because cardiac denervation eliminates efferent control of heart rate indefinitely (i.e. reinnervation in humans is unsubstantiated) (Rowan and Billingham, 1988; Regitz et al., 1990), long-term decrements in exercise capacity have typically been attributed to reduced chronotropic reserve (Schroeder, 1979; Savin et al., 1980; Pope et al., 1980; Pflugfelder et al., 1987; Nixon et al., 1989; Colucci et al., 1989; Quigg et al., 1989).

Although efferent denervation diminishes chronotropic reserve, dynamic exercise generates a variety of neural and humoral compensatory responses to maintain systemic blood pressure and cardiac output in the presence of cardiac denervation. The sympathetic nervous system directly maintains cardiac output through peripheral arteriolar vasoconstriction in nonworking muscles, the splanchnic and renal circulations as well as via the venoconstriction of the capacitance vessels (Sagawa, 1983; Rowell and O'Leary, 1990). Indirectly, the sympathetic nervous system augments the heart rate response through the effects of circulating catecholamines (Rowell, 1986). In HTR, additional humoral compensation in heart rate reserve occurs through hypersensitivity to circulating catecholamines (Bexton et al., 1983; Lurie et al., 1983; Vatner et al., 1985; Yusuf et al., 1987; Gilbert et al., 1989; Port et al., 1990; Bristow, 1990; Regitz et al., 1990). Also, parameters of diastolic function and contractile reserve are normal in some HTR which suggests that circulating catecholamines may also have a very important inotropic influence during dynamic exercise (McLaughlin, 1978; Pope, 1980; Borow, 1985a). In summary, Tx alters the heart rate response to exercise but compensatory mechanisms exist. In some HTR, comparably good exercise performance can be obtained in the absence of

normal efferent autonomic control of the heart. The denervated heart appears to function adequately during exercise with the possible exception of exercise situations that require an immediate tachycardic response.

Justification for Further Research

Recent clinical and experimental evidence suggests that physiological mechanisms other than efferent denervation may underlie the exercise intolerance in HTR. Neuroendocrine abnormalities resulting from cardiac deafferentation (Zambraski et al., 1984; Mohanty et al., 1987; Myers et al., 1988), cyclosporine induced pulmonary gas exchange abnormalities (Casan et al., 1987) and skeletal muscle weakness (Kavanagh et al., 1988) are recent postulates for the unexplained exercise intolerance. The available data regarding these mechanisms, however, is sparse and conflicting and the physiologic nature of diminished exercise capacity in HTR remains unclear. Therefore, further studies of medically stable HTR involving measurements of muscular strength and cardiac, pulmonary and neuroendocrine responses to exercise are necessary to elucidate the mechanisms most responsible for diminished exercise capacity.

Cardiac Deafferentation and the Neuroendocrine Response to Exercise

Exercise capacity may be altered by the systemic effects of the cardiac deafferentation associated with Tx (Thames et al., 1971; Zambraski et al., 1984; Mohanty et al., 1987; Myers et al., 1988). When the heart is transplanted, the efferent nerves passing to the donor heart as well as the afferent or sensory nerves that originate in the stretch receptors of the donor heart are surgically ablated (Baumgartner et al., 1990). When intact,

the sensory receptors send afferent signals to the vasomotor control center in the medulla (brain stem) via the vagal nerve. Sensory input from the cardiac baroreceptor reflexes plays an important regulatory role in the neural and hormonal control of circulation. Under normal circumstances, sensory receptors in the atria and ventricles exert a tonic inhibitory influence on sympathetic nervous activity to the heart and peripheral circulation as well as exerting a restraining influence on the circulating levels of pressor hormones such as norepinephrine (NE), epinephrine (E), arginine vasopressin (AVP) and the renin-angiotensin-aldosterone system (RAAS) (Paintal, 1953; Johnson et al., 1969; Ledsome and Mason, 1972; Mancina and Donald, 1975; Quillen and Cowley, 1983).

In congestive heart failure (CHF) patients awaiting Tx, the function of cardiac baroreflexes is profoundly impaired (Chidsey et al., 1962; Thomas and Marks, 1978; Dzau et al., 1981; Goldsmith et al., 1983a). Overt CHF is characterized by stimulation of several neuroendocrine systems involved in electrolyte balance and blood pressure homeostasis. Atrial natriuretic peptide (ANP), NE, E, AVP and RAAS in particular, are elevated in CHF (Goldsmith et al., 1983a; Raine et al., 1986; Colucci et al., 1989; Francis et al., 1990; Swedberg et al., 1990). Tx restores cardiac function and reverses CHF but it is uncertain how Tx affects the neurohumoral excitatory state associated with CHF. Tx in humans has been reported to increase (Banner et al., 1989; Scherrer et al., 1990) and decrease (Thames et al., 1971; Mohanty et al., 1987; Banner et al., 1990) reflex elevations of plasma NE. At this juncture, however, the consequences of Tx in humans are not clearly understood. More specifically, little is known regarding the effects of possible deafferentation on the circulating levels of other vasoactive

hormones during exercise. Exercise alters the release of AVP, ANP, NE and RAAS but the neuroendocrine response to exercise in HTR has not been investigated. The denervated heart is more reliant upon the Frank Starling mechanism to augment cardiac output and consequently, there is an increased dependence upon cardiac preload reserve (Pope et al., 1980; Pflugfelder et al., 1987; Younis et al., 1990). It is unknown, however, if compensatory mechanisms responsible for maintaining blood pressure and cardiac output during exercise are stimulated in HTR. Thus, determination of neuroendocrine responses during various levels of exercise is critical to the resolution of this issue. A more precise description of the temporal pattern of plasma AVP, NE, RAAS and ANP in HTR during steady-state and non-steady state exercise will offer a more complete understanding of the compensatory mechanisms regulating cardiac output following Tx.

Immunosuppression, Denervation and the Pulmonary Response to Exercise

Tolerance for aerobic exercise in HTR may be limited by inadequate pulmonary gas exchange, specifically gas exchange during the rest to exercise transition. Abnormal pulmonary diffusion capacity (DLCO)(Casan et al., 1987; Banner et al., 1988) and sluggish pulmonary ventilation ($\dot{V}_E$) at the onset of exercise (Marconi et al., 1987; Meyer et al., 1989) have been reported following Tx.

The nature of DLCO impairment in HTR is unknown. Immunosuppression therapy with cyclosporine, the surgical procedure and reduced hemoglobin have been cited as possible mechanisms responsible for diminished DLCO (Casan et al., 1987; Wright et al., 1990;

Bussieres et al., 1990). However, because DLCO is markedly impaired in CHF patients awaiting Tx (McNeil et al., 1958; Morley et al., 1989; Wright et al., 1990; Bussieres et al., 1990), it is difficult to conclude that abnormal DLCO postTx is due to immunosuppressive agents, the surgical procedure or simply the persistence of pre-existing abnormalities (eg. CHF, chronic obstructive pulmonary disease etc.). Unfortunately, studies that have measured pulmonary function in the same patient sample before and after Tx and accounted for differences in age, smoking history, disease severity and months postTx are noticeably absent from the literature.

Casan et al. (1987) compared pre- and postTx pulmonary function in HTR (n=10) and found improved lung volumes and flow rates but a 14% deterioration of DLCO. The authors summarized their finding of reduced DLCO by stating that a good correlation ($r = 0.87$) existed between the cyclosporine level in whole blood and the degree of reduction in DLCO. They suggested that cyclosporine may induce interstitial infiltration and vascular endothelial cell proliferation similar to what is observed in the kidneys of renal transplant patients receiving high doses of this drug (Myers et al., 1984; Taube et al., 1985). These results, however, have not been replicated, and little evidence exists to suggest that induction of immunosuppression with cyclosporine may be complicated by lung dysfunction. Furthermore, most of the existing pulmonary function data involving HTR has been collected during resting conditions. It remains to be determined whether HTR who exhibit reduced pulmonary diffusion capacity at rest will also experience transient arterial hypoxemia during dynamic exercise. Additional research is necessary to determine resting

pulmonary function and its influence on arterial blood gasses in patients before and after Tx during both rest and exercise conditions.

Another explanation for inadequate pulmonary gas exchange at the onset of exercise in HTR has been proposed by Wasserman and coworkers (Wasserman et al., 1974; Uchida, 1976; Jones et al., 1982). They found highly correlated, linear relationships ($r=0.94$) between expired minute ventilation ($\dot{V}_E$) and changing right ventricular load in experimental animals. Because the same relationship existed when right ventricular load was increased independent of cardiac output (by balloon obstruction), the authors concluded that a neural pathway originating from right ventricular stretch receptors may stimulate ventilation at the onset of exercise ("cardiodynamic hyperpnea"). The neural pathway for the afferent limb of this reflex has not been identified in animals, and we are unaware of other data to support the contention that cardiac stretch receptor signals may be a stimulus for exercise hyperpnea in man. Denervated HTR provide a unique opportunity to study the "cardiodynamic hyperpnea" hypothesis. Serial arterial blood gas measurements (PaO_2 ; PaCO_2 ; pH) during the transition from rest to steady-state exercise would determine the ability of HTR to achieve necessary ventilation and provide adequate gas exchange during the onset of exercise as well as ascertain whether the ventilatory response is dependent upon the cardiac output response.

Disuse Atrophy, Steroid Therapy and Muscular Strength

A non-cardiopulmonary explanation for diminished exercise capacity in HTR may be skeletal muscle atrophy. Prolonged preoperative inactivity due to severely limited cardiac output and postoperative corticosteroid

treatment (eg. prednisone) for immunosuppression appears to adversely affect strength and lead to skeletal muscle atrophy (Horber et al., 1985; Kavanagh et al., 1988; Squires, 1990). Corticosteroids have been shown to cause pronounced atrophy of skeletal muscle ("steroid myopathy") when administered at high doses for prolonged periods (Goldberg and Goodman, 1969; DuJovne and Azarnoff, 1975; Kinney and Felig, 1979; Almon and Dubois, 1990). Kavanagh et al. (1988) recently found that the body weight of HTR was lower than those of age matched controls and that the difference in body weight was largely attributable to decreased lean mass. The authors speculate that increases in peak heart rate and exercise capacity in HTR participating in endurance exercise training could largely be due to a strengthening of the leg muscles. Weight gain frequently follows Tx (Keteyian, 1989) but a better understanding of the changes which occur in specific body compartments (ie. lean body mass vs fat tissue) postoperatively would assist with long-term weight management. Additionally, there is evidence that muscle atrophy from prednisone can be prevented or attenuated by exercise training (Seene and Viru, 1982; Horber et al., 1985; Hickson et al., 1986; Czerwinski et al., 1987; Horber et al., 1987). To date, however, no data are available concerning the influence of exercise training on muscular strength development in HTR. Furthermore, the muscular strength of HTR has not been quantified with a standardized criterion strength test and compared with normal control values.

Purpose of the Study

Thus, there are three critical questions which will be addressed regarding the etiology of the diminished exercise capacity observed in most HTR: 1) Does Tx alter the neuroendocrine response during exercise? 2) Does Tx and subsequent immunosuppression therapy adversely affect minute ventilation, DLCO and arterial oxygenation? and 3) What role does muscle disuse atrophy and possible steroid myopathy play in limiting exercise capacity?

The present study is designed to investigate these three questions which relate to mechanisms that may independently or collectively act to diminish exercise capacity in HTR. Specifically, the purposes of this study are 1) to determine the temporal pattern of neuroendocrine responses (AVP, NE, RAAS, ANP) and cardiac hemodynamics (heart rate, stroke volume, cardiac output) during the transition from rest to steady-state and non-steady state exercise; 2) to determine the temporal pattern of blood gasses (PaO_2 , PaCO_2 , pH) during the transition from rest to steady-state and non-steady state exercise, and to compare resting pulmonary mechanics and DLCO before and after Tx; and 3) to quantify bilateral knee extension (quadriceps) strength.

Hypotheses

When examining the test results of HTR and control subjects matched with respect to age, gender and body composition:

- 1) there will be no significant difference in neuroendocrine responses during maximal and submaximal cycle exercise;

- 2) there will be no significant difference in arterial blood gas responses during submaximal cycle exercise;
- 3) there will be no significant difference in cardiodynamic responses during submaximal cycle exercise;
- 4) there will be no significant difference in resting pulmonary function; and
- 5) there will be no significant difference in knee extension leg strength.

When examining the test results of orthotopic heart transplant patients before and after transplantation:

- 1) there will be no significant difference in resting pulmonary function.
- 2) there will be no significant difference in arterial blood gasses.

Delimitations

This study was delimited to the following:

- 1) eleven volunteer HTR without clinical or endomyocardial biopsy evidence of significant rejection, infection or other contraindications for exercise;
- 2) eleven healthy, non-athletic and non-medicated control subjects that matched the HTR with respect to gender, age and body size;
- 3) subjects that ranged in age from 21 to 63 years;
- 4) HTR that were 7 to 41 months posttransplantation;

- 5) transplant patients receiving immunosuppressive therapy with cyclosporine, azathioprine and prednisone;
- 6) persons not receiving cardiac beta-receptor blocking drugs; and
- 7) persons consenting not to change diet, prescription medications or physical activity habits during the course of the study.

Limitations

This study was limited by the following:

1. The HTR were receiving antihypertensive medication that may influence the cardiovascular and neuroendocrine responses to exercise.
2. The HTR were not all receiving the same antihypertensive medications.
3. Pre- and posttransplantation pulmonary function tests in the HTR were not performed in the same laboratory.
4. Cardiac output was estimated by Electrical Bioimpedance Cardiography.
5. Hemoglobin and hematocrit were obtained with a Centrifugal Hematology System.
6. Only knee extension (quadriceps) strength was evaluated.

CHAPTER 2

REVIEW OF THE LITERATURE

Due to the nature of this investigation, the review of literature will be divided into three major sections. The first section will review the research that deals with neuroendocrine responses at rest and during exercise in HTR and during volume reduction and expansion in cardiac denervated animals. The second portion of the review will evaluate current research of pulmonary function following Tx. The third section will review the available research that describes the skeletal muscular strength of HTR postoperatively.

Neuroendocrine Hormones

Norepinephrine

Norepinephrine (NE) is the neurotransmitter released at postganglionic synapses of the sympathetic nervous system (SNS). In addition, the adrenal medulla secretes epinephrine (E) and NE in a ratio of approximately 4:1 (Guyton, 1986). The adrenal medulla, however, may be considered a "special case" within the SNS. Instead of directly secreting neurotransmitter onto end organs, the adrenal medulla secretes into the bloodstream and thus has profound actions at a distance. Nonetheless, the main source of plasma NE is spillover of the hormone released from the axon terminals of sympathetic neurons (Goldstein, 1981; Christensen and Galbo, 1983). In normal humans, the myocardium, sinoatrial (SA) node and coronary vessels are richly innervated by the SNS. Because Tx severs

sympathetic nerve fibers and causes total cardiac denervation, the local delivery of NE to the heart by sympathetic nerves is either greatly reduced or entirely absent (Rowan and Billingham, 1988; Regitz et al., 1990). Therefore, beta-adrenergic receptor stimulation of the heart is solely due to circulating plasma NE and E. Although a number of other substances are coreleased with E and NE (eg. dopamine, chromogranin A), plasma NE levels are generally regarded as the best humoral marker of global SNS activity in response to exercise (Goldstein, 1981). Evidence to support the use of plasma NE as an index of SNS activity comes from recent microelectrode studies which have demonstrated high correlations ($r=0.72$ to 0.81) between antecubital venous NE levels and the number of action potentials recorded during direct monitoring of peripheral sympathetic nerves (Leimbach et al., 1986; Victor et al., 1987; Seals et al., 1988).

Norepinephrine Release During Exercise in HTR. The purpose of this portion of the literature review is to examine the available evidence regarding SNS activity and the plasma NE response during exercise in HTR. Resting SNS activity is increased in patients with CHF (Goldsmith et al., 1983a; Raine et al., 1986; Colucci et al., 1989; Francis et al., 1990; Swedberg et al., 1990). Tx restores cardiac function but it is uncertain whether Tx eliminates sympathetic overactivity. Specifically, little information is available regarding the SNS activity during exercise in HTR.

One of the earliest reported studies of the exercise response of the denervated human heart included measurements of plasma NE (Pope et al., 1980). The effects of supine multistage exercise on a cycle ergometer (3 minutes at 15, 45 and 90 watts) were assessed in 6 HTR. Plasma NE increased slowly from 233 pg/ml at rest to 460 at 45 watts and then rapidly

increased to 1,970 pg/ml at 90 watts (6 fold increase from rest). Increases in contractility (defined by the authors as velocity of circumferential fiber shortening) and heart rate were highly correlated with increasing NE concentrations ($r=0.92$ and $r=0.79$, respectively). Unfortunately, however, this study did not include a control group, so it is difficult to infer whether the NE levels elicited by these work intensities represent sympathetic overactivity or an appropriate response. Moreover, comparisons with more recent HTR studies that utilize upright exercise are difficult because of the fact that lower NE levels are present during supine exercise than in the upright position (Dimsdale and Ziegler, 1991). Nonetheless, these data are useful in that they were the first to demonstrate the relationship between humoral adrenergic mechanisms and the adaptation of the transplanted human heart to vigorous exercise. Confirmation of this relationship was shown by others who found that beta blockade greatly attenuated the heart rate response to exercise in HTR, indicating that humoral catecholamines contribute to the response of the denervated heart (Bexton et al., 1983). Moreover, the issue of inotropic support from circulating catecholamines has recently been clarified by Port et al. (1990), who found that the transplanted human heart contains an unexpectedly high percentage and density of beta adrenergic receptors which suggests that circulating NE should provide inotropic stimulation.

More recently, Degre et al. (1987) recorded plasma NE levels in 15 HTR at rest and during a SL-GXT on an upright cycle. Plasma NE was 158 pg/ml at rest and reached 2,060 pg/ml immediately after exercise. Although the study did include a control group, NE data were not reported for the control subjects, so conclusions regarding the relative level of SNS activity

are difficult to make. Furthermore, HTR in this study were only 38 ± 10 days postTx which may have been too early to detect the effects of deafferentation on cardiac and neuroendocrine reflexes that maintain blood pressure homeostasis. It has been shown that the plasma NE response to exercise is significantly greater than control in HTR studied > 3 months after Tx but NE levels were not different from control in patients studied < 3 months after Tx (Quigg et al., 1989).

Quigg et al. (1989) measured levels of plasma NE at rest and at peak exercise on an upright cycle ergometer in 23 HTR an average of 7 ± 1 months after Tx and in 23 control subjects matched for age. HTR were grouped into those studied within 3 months of Tx ("early recipients"; $n=11$) and those studied more than 3 months after Tx ("late recipients"; $n=12$). Plasma NE at rest (324 vs 255 pg/ml) and peak exercise (1,972 vs 1,653 pg/ml) was similar ($p \geq 0.05$) for early HTR and control subjects, respectively. In late HTR, however, plasma NE at rest (452 vs 255 pg/ml), peak exercise (2,886 vs 1,653 pg/ml) and the increment from rest to peak exercise (2,434 vs 1,398 pg/ml) was significantly ($p \leq 0.05$) increased compared with control subjects. These data suggest that the sympathetic response to peak exercise is increased in late HTR but the explanation for this trend is not clear.

Banner and coworkers (1989) also measured plasma NE levels in 7 HTR during a SL-GXT on an upright cycle ergometer. Compared to age and sex matched control subjects, HTR had higher NE levels at rest (3.9 vs 2.9 nmol/L) and greater ($p \leq 0.05$) relative increments of NE at each submaximal exercise interval successfully completed by both groups. Peak NE concentrations of HTR were similar ($p \geq 0.05$) to those of the control group. The authors conclude that the increased circulating levels of NE

during submaximal exercise may be due to surgical deafferentation as well as reduced peripheral vascular responsiveness. Although each of these hypotheses offer a viable explanation for the observed sympathetic overactivity, an alternative interpretation of their data may be indicated. Considering the fact that $\dot{V}O_{2\max}$ and peak power output achieved by HTR were only 60% of that achieved by the control group, each submaximal exercise stage would have required a higher relative percentage of maximal effort in HTR. Therefore, the observed increased sympathetic response during exercise may only be indicative of the well documented positive linear relationship between relative exercise intensity and SNS responsiveness (Chidsey et al., 1962; Howley, 1976; Goldstein, 1981; Christensen and Galbo, 1983; Rowell and O'Leary, 1990). To date, however, no studies have been performed which compared plasma neuroendocrine responses in HTR and matched control subjects during exercise at the same relative levels of exercise intensity.

Younis et al. (1990) studied hemodynamic responses in 10 HTR during upright cycle exercise at three levels of relative intensity (50, 70 and 90% of peak power output). Plasma NE was measured at baseline and after each 5 minute submaximal exercise test. Plasma NE levels averaged 456 pg/ml at rest and rose to 1312 pg/ml at the second level of exercise and to 2157 pg/ml at the third level of exercise. Unfortunately, it is difficult to draw conclusions from these data regarding relative levels of SNS activity because control NE data were not reported.

Scherrer and associates (1990) recently provided more direct evidence of SNS overactivity in HTR. They performed microelectrode recordings of efferent sympathetic action potentials in the peroneal nerve of HTR. The

interesting feature of their data was that cyclosporine immunosuppression appeared to be associated with increased SNS activity and hypertension. HTR receiving cyclosporine (n=10) had higher ($p \leq 0.05$) mean arterial blood pressure than HTR not receiving cyclosporine (n=5) (112 vs 96 mmHg) and a 2.7-fold higher rate of sympathetic nerve firing (80 vs 28 bursts/min). Plasma NE levels were higher in HTR who received cyclosporine than in those who did not take cyclosporine or in normal control subjects (262, 174 and 152 pg/ml, respectively). Although these data demonstrate the possible sympathoexcitatory effect of cyclosporine, cardiac deafferentation may have enhanced the observed effect. The investigators compared the effects of cyclosporine in HTR with normally innervated myasthenia gravis patients receiving immunosuppressive therapy with cyclosporine. Similar doses of cyclosporine were associated with significantly ($p \leq 0.05$) larger increases in SNS activity in HTR than in the myasthenia gravis patients (46 vs 25 bursts/min).

In addition to exercise, head-up tilt has been used to test the integrity of cardiovascular control mechanisms in HTR. Passive head-up tilt reduces stroke volume by reducing cardiac preload and stroke volume. This activates cardiac and arterial baroreceptor reflexes, resulting in increased heart rate and contractility with peripheral vasoconstriction and associated neuroendocrine activity as compensatory measures to maintain blood pressure and cardiac output. Banner and coworkers (1990) reported normal baseline NE levels but increased NE responses to head-up tilt in 8 HTR. After 60 minutes of head-up tilt, the decrease in cardiac output (30% vs 18%) and the increase in systemic vascular resistance (52% vs 28%) were greater ($p \leq 0.05$) in HTR. These data indicate that SNS overactivity was

necessary to maintain blood pressure and cardiac output in HTR. The authors speculate that increased SNS activity may be due to an increased activation of arterial baroreceptor reflexes related to the greater decrease in cardiac output or due to the loss of tonic inhibition of the SNS following cardiac deafferentation.

Not all studies have reported normal and/or elevated SNS activity in HTR. Mohanty and coworkers (1987) studied cardiovascular control mechanisms in 23 HTR by measuring plasma NE, blood pressure and forearm vascular resistance during lower-body negative pressure (LBNP). This technique (at mild suction) unloads cardiac rather than arterial stretch receptors, which typically results in peripheral vasoconstriction and increased levels of circulating NE. They reported that heart rate, mean arterial pressure and forearm vasoconstriction did not respond during application of LBNP (at -10, -20 and -40 mmHg) and the NE response was attenuated in HTR compared to the control group. The authors concluded that the likely mechanisms for the impairment in reflex control of the circulation was cardiac deafferentation. These results are not in opposition to the findings in denervated subjects during exercise because LBNP is a volume reduction intervention whereas exercise increases atrial stretch. It is curious, however, that significantly lower resting plasma NE levels were unique to this group of HTR.

In summary, circulating NE has been shown to have important chronotropic and inotropic effects upon the chronically denervated human heart and provide a measure of compensation for denervation in HTR. The degree of SNS overactivity during exercise, however, cannot be

ascertained from the available data because control and HTR were not studied at similar relative exercise intensities.

Arginine Vasopressin

Arginine vasopressin (AVP) is a nine amino acid polypeptide released from the nerve endings of the posterior pituitary gland (Guyton, 1986). The primary physiological functions of AVP are antidiuresis and peripheral vasoconstriction (Johnson et al., 1969; Ledsome and Mason, 1972; Cowley et al., 1974; Cowley et al., 1984). Extremely minute quantities of AVP can cause antidiuresis. In the presence of AVP, the permeability of renal collecting ducts and tubules to water increases greatly and allows most of the water to be reabsorbed, thereby conserving water in the body (Guyton, 1986). Vasopressin at higher plasma concentrations is also a potent vasoconstrictor in man. When AVP is present in moderate and high concentrations, AVP receptors in the arterioles mediate vasoconstriction in those vessels and thereby play an important role in regulating blood pressure and cardiac filling pressure (Cowley et al., 1974; Quillen and Cowley, 1983; Manning and Sawyer, 1984).

Early research generally concluded that the normal plasma levels of AVP (1-2 pg/ml) were too small to exert a significant pressor effect and participate in the normal daily blood pressure regulation (Sawyer, 1961). Several noteworthy design flaws, however, may have masked the pressor effect of AVP. Arterial baroreflex compensation, sodium pentobarbital anesthetic and stress induced saturation of the system with endogenous AVP may have altered vascular reactivity during AVP infusion studies (Cowley et al., 1974). Subsequent studies which controlled for the compensatory role of the arterial baroreflexes and used unanesthetized,

undisturbed animals have demonstrated that between 5 and 10 mm Hg of the normal arterial pressure is maintained by basal levels of AVP. Furthermore, it has been shown that when arterial pressure or left atrial pressure fall, the reflex secretion of AVP is enough to bring mean arterial blood pressure three fourths of the way back to normal (Cowley et al., 1974).

Vasopressin is released into the circulation through activation of hypothalamic "osmoreceptors". The osmoreceptors, in turn, stimulate adjacent neurons in the supraoptic and paraventricular nuclei of the hypothalamus which have been shown to be involved in AVP synthesis (Ledsome and Mason, 1972; Quillen and Cowley, 1983).

In addition to osmoregulation, there is a large body of evidence which argues that AVP release is mediated by low-pressure stretch receptors in the left atrium which send their afferent signal via the vagal nerve. Perhaps the most powerful stimuli for increasing the secretion of AVP is loss of blood volume or decreased left atrial filling pressure (Quillen and Cowley, 1983). The increased secretion of AVP is believed to result mainly from the low atrial pressure in the heart caused by the low blood volume and resultant decrease in cardiac preload. The unloading of the atrial stretch receptors removes tonic inhibition of ANP and evokes the reflexive increase in AVP secretion (Johnson et al., 1969; Ledsome and Mason, 1972; Mancina and Donald, 1975; Quillen and Cowley, 1983). However, the carotid and aortic baroreceptors also participate in the modulation of AVP secretion (Cowley et al., 1974; Cowley et al., 1984).

The possibility that the heart influences the secretion of AVP was suggested 35 years ago by Henry et al. (1956), who noted that urine flow increased when the left atrium of an anesthetized dog was distended.

Furthermore, this response was eliminated when the cervical vagus nerves were cooled to block neural transmission. Henry and coworkers (1956) hypothesized that stretch receptors in the left atrium reflexly inhibited the release of AVP and thereby increased diuresis. The diuretic response to balloon distension of the left atrium was used by several investigators to support the hypothesis that cardiac receptors play an important role in the control of AVP secretion (Gauer and Henry, 1963; Ledsome and Mason, 1972). However, these findings were confounded by the discovery that left atrial receptors also modify sympathetic outflow to the kidney and could change urine volume through this mechanism independent of AVP release (Karim et al., 1972; DiBona, 1978). Subsequently, it was confirmed that left atrial stretch increased atrial volume receptor firing (type B receptors) and inhibited AVP release in dogs (Johnson et al., 1969; Ledsome and Mason, 1972). Numerous studies using balloon inflation or fluid infusion to distend the left atria have reported decreased plasma AVP in response to either stimulus. In contrast, right atrial distension did not decrease plasma AVP levels. More recently, Wang et al. (1988) demonstrated that ventricular receptors may also be involved in mediating AVP release. Conversely, reduction of left atrial stretch through hemorrhage or other means of volume contraction was associated with an increase in plasma AVP.

These responses, however, have not always been demonstrated in anesthetized subhuman primates under similar circumstances. Gilmore and Zucker (1978) hypothesized that the sensitivity of the reflex diminished during the evolution to the upright posture of the primate and

thereby help maintain blood pressure homeostasis in a primate that frequently changes between the upright and the supine position.

AVP Release During Exercise. Atrial pressure increases during exercise but, despite this inhibitory influence, plasma AVP is increased in a dose-response relationship with increasing exercise intensity. Exercise alters a variety of factors that are known to affect the secretion of AVP. Plasma osmolality, angiotensin II and cortisol are elevated with exercise while plasma volume decreases due to the outward movement of fluid from the vascular space to the interstitial space. The outward movement of fluid from the vascular space to the interstitial space during exercise is dependent upon the intensity of the exercise (Wilkerson et al., 1977). The resulting decrease in plasma volume has been associated with proportional increases in electrolyte and protein concentrations. All of the above factors have been reported to stimulate AVP release from the pituitary gland (Wilkerson et al., 1977; Convertino et al., 1981; Wade and Claybaugh, 1980; Geyssant et al., 1981).

Available evidence indicates that an acute bout of exercise does result in an increase in plasma AVP (Convertino et al., 1981; Wade and Claybaugh, 1980; Geyssant et al., 1981). The magnitude of AVP release appears to be related to the relative work intensity as measured in terms of maximum oxygen consumption (Wade and Claybaugh, 1980; Convertino et al., 1981; El-Sayed, 1990). Convertino et al. (1981) reported nonsignificant changes in plasma AVP at 40% of $\dot{V}O_2$ max but a 5 fold increase in AVP at 70% of $\dot{V}O_2$ max and 12 fold increases in AVP at maximal exercise. El-Sayed (1990) found 10-fold increases in AVP at maximum effort during incremental cycle graded exercise tests. These data suggest that a threshold

work intensity above 40% of $\dot{V}O_2$ max may be required to increase plasma AVP concentrations during exercise but sufficient data is lacking to confirm this relationship.

Factors mediating the release of AVP during exercise are not fully understood. The acute reduction in plasma volume that accompanies even short term exercise has been suggested as one mechanism responsible for elevated AVP (Geyssant et al., 1981). Convertino and associates (1981) suggested that the hyperosmolality evoked by transient hemoconcentration, rather than reduction in plasma volume, results in AVP release during exercise. Landgraf et al. (1982) also reported that plasma osmolality changes were significantly correlated with AVP responses during exercise. Others have presented conflicting data which indicates that AVP may not be mediated primarily by osmolality changes. Beardwell and coworkers (1975) and Beaumont et al. (1973) have shown that the AVP response after exercise was greater than that found after dehydration despite the fact that osmolality changes were similar in response to both conditions.

AVP Release In Cardiac Denervated Animals. The reflex inhibition of AVP release during distension of the left atria is absent in animals with chronic cardiac denervation (Fater et al., 1982). Therefore it is conceivable that mechanisms which elevate AVP during exercise are unapposed in HTR and the result is a disproportionate elevation in AVP when compared to innervated controls.

The increase in plasma AVP during hemorrhage is markedly attenuated in dogs with chronic cardiac denervation (Wang et al., 1983). Central volume unloading during head-up tilt and frusemide infusion in

cardiac denervated humans also elicits a blunted AVP response (Banner et al., 1990; Drieu et al., 1986).

Wang et al. (1983) measured plasma AVP and PRA during continuous hemorrhage in cardiac denervated and sham-operated conscious dogs. Baseline AVP concentrations were not different ($p \geq 0.05$) between the control (3.8 pg/ml) and cardiac denervated dogs (3.3 pg/ml). Mild hemorrhage (10 ml blood/kg body weight) caused a significant increase in plasma AVP in control dogs but not in cardiac denervated dogs. The increase in AVP during hemorrhage of 20 and 30 ml of blood/kg body weight was attenuated in the cardiac denervated animals when compared to the response of the control dogs. The mean concentration of AVP in the cardiac denervated dogs was only 10-15% as elevated as in the control dogs (33 vs 300 pg/ml).

AVP Release In HTR. Several studies have measured resting plasma AVP in HTR but the relationship between exercise intensity and AVP response has not been reported in HTR.

Mertes et al. (1991) followed 10 HTR for a period of 10 days following surgery. Mean preoperative AVP levels were found to be elevated (7.5 pg/ml). The postoperative changes in AVP were marked by a secretory peak occurring on the first day of recovery (87 pg/ml). These elevated levels decreased but remained slightly above normal through the 10th day of recovery (4.9 pg/ml). The authors attributed the secretory peak, in part, to the surgical stress of Tx. Similar findings have been reported by other investigators who have measured AVP release during and following cardiopulmonary bypass in humans (Viinamaki et al., 1986) and following anaesthesia and surgery in dogs (Bonjour and Malvin, 1970; Simon-

Oppermann et al., 1987). Viinamaki et al. (1986) reported that AVP increased nearly 6-fold in human cardiac surgery patients (n=32) after 5 to 10 minutes of cardiopulmonary bypass and remained significantly increased during the first postoperative day but returned to preoperative levels by the 4th postoperative day. Mean arterial blood pressure had also decreased to its nadir 5-10 minutes after induction of cardiopulmonary bypass, therefore the authors concluded that the mechanism leading to AVP release was likely via baroreceptor activation.

Banner et al. (1990) studied neuroendocrine responses to one hour of 45° head-up tilt in 8 recipients of combined heart-lung transplantation. Baseline AVP concentrations were similar in the transplant and control groups (2.6 vs 2.5 pmol/l), and the authors report that AVP levels did not increase significantly during head-up tilt in either group. Inspection of their AVP data, however, reveals that there was a trend toward elevated AVP in HTR with AVP reaching plasma concentrations at 60 minutes of tilt that were approximately 1.6 times greater than baseline whereas AVP did not change in the control group. Because heart rate, systolic and diastolic responses to tilt were the same in both groups, the authors concluded that afferent information from cardiac stretch receptors and efferent cardiac innervation were not essential to maintain systemic blood pressure under orthostatic stress. However, the trend toward elevated AVP suggests that blood pressure homeostasis in HTR involved higher AVP secretory rates perhaps made possible by the deafferentation of inhibitory cardiac atrial fibers.

Drieu and coworkers (1986) studied the plasma AVP and PRA response to volume depletion with frusemide and volume expansion with

water load in 13 HTR. A baseline AVP and PRA sample was drawn after 30 minutes of supine rest. Lasix (40 mg) was then injected intravenously and the subjects walked casually for 60 minutes after which AVP and PRA samples were again drawn. Basal plasma AVP was significantly elevated in HTR compared to 10 healthy control subjects (4.5 vs 3.1 pg/ml). During lasix induced volume depletion, however, AVP concentrations did not respond in HTR (4.5 and 4.7 pg/ml before and after lasix) but increased 45% from baseline in the control group. Resting PRA was not different between groups (1.7 vs 1.2 ng/ml/hr for the control and HTR, respectively) and the response to volume depletion was similar ($p \geq 0.05$). These results are consistent with the findings after volume depletion via hemorrhage in cardiac denervated dogs (Wang et al., 1983). It remains to be determined, however, what the AVP response will be in HTR during increased atrial pressure while exercising. Higher plasma AVP is typically observed under basal conditions in HTR and this may be attributed to the absence of tonic inhibition of AVP release by the cardiac stretch receptors.

In summary, plasma AVP is released in a dose-response relationship with increasing exercise intensity. Factors mediating the release of AVP during exercise are acute plasma volume reduction, transient hemoconcentration and SNS stimulation. However, no data are available on the possible modification of the AVP response during exercise in HTR.

Atrial Natriuretic Peptide

The discovery of secretory granules in rat atrial myocytes by de Bold et al. (1981) has led to the isolation of a new circulating hormone, atrial natriuretic peptide (ANP). Its biologically active sequence is composed of 28-amino acids and has been shown to have diuretic, natriuretic and

vasorelaxant properties (deBold et al., 1981). These findings indicate that the heart may have an endocrine function in electrolyte balance and blood pressure homeostasis that is usually associated with the RAAS and SNS.

Although hundreds of studies on ANP have been performed since its discovery 10 years ago, the role of ANP in the physiologic regulation of the cardiovascular system remains controversial. Isolated aorta and renal vessels partially constricted with a variety of pressor agents have been shown to relax in the presence of ANP (Goetz, 1988). Conflicting data, however, have been obtained for other arteries and veins (Goetz, 1988). Moreover, vasodilation does not always occur when ANP is given to intact animals. Studies on conscious animals have demonstrated that peripheral resistance may remain constant or even increase when ANP is infused (Goetz, 1988). The explanation for this response to ANP during *in vivo* experiments, however, may be that arterial baroreceptors respond to the hypotensive effects of ANP and elicit reflex increases in sympathetic vasoconstriction, thereby offsetting any vasodilation induced directly by the ANP.

Human subjects respond somewhat differently than animals when given ANP infusions. ANP causes cutaneous vasodilation and human subjects occasionally develop facial flushing and hypotension with the decrease in blood pressure being severe in some cases. It is apparent that ANP can be a potent vasodilator in normal human subjects. Nearly all reports indicate that a decrease in blood pressure elicited by ANP in humans is accompanied by an increase in heart rate (Weidmann et al., 1986; Goetz, 1988). Presumably, the increase in heart rate is mediated via a reflex initiated by the arterial baroreceptors.

ANP is also capable of decreasing plasma volume (Weidmann et al., 1986). Although a reduction in plasma volume obviously would be expected (over time) in response to the diuretic and natriuretic properties of ANP, plasma volumes are also reported to diminish in anephric animals that are given ANP. Comparable results have been obtained after removal of both kidneys and spleen (Goetz, 1988). Goetz (1988) suggests that the ability of ANP to cause a net transfer of fluid from plasma to interstitial space raises the possibility that edema formation in CHF and other conditions associated with abnormally high plasma ANP levels (i.e., Tx) may be attributable in part to the effects of ANP.

Infusions of ANP that raised the plasma concentration of ANP 3-7 and 12 fold in conscious dogs caused progressive decreases in right and left atrial pressures (Goetz, 1988). Further work is needed to establish that the effects of ANP on arterial pressures are of physiologic importance. However, because an increase in atrial pressure increases the release of ANP, which in turn may decrease cardiac filling pressure, the atrial peptides may serve as part of a negative feedback system that enables the heart to influence its own filling pressures (Goetz, 1988). The decrease in cardiac filling pressure or preload appears to be primarily responsible for the decrease in cardiac output that may be induced by ANP (Goetz, 1988).

In isolated tissue preparations, ANP blocks the vasoconstrictor effects of NE and ANG II. In normal healthy humans, elevated ANP appears to exert an inhibitory effect upon the RAAS (Shenker et al., 1985). This inhibitory effect of ANP upon the RAAS, however, has not been observed in some populations. Inhibition of the RAAS, for example, has not been demonstrated in CHF patients (Shenker et al., 1985; Cody et al., 1986).

Notwithstanding these exceptions, ANP is thought by some investigators to be an important counter-regulatory hormone to the RAAS. Thus, it has been hypothesized that ANP may be a potentially important hormone in blood pressure homeostasis. Specifically, elevated ANP may be a physiologic response to protect the system by reducing afterload in hypertension or afterload and preload in heart failure.

There is evidence to suggest that high plasma levels of ANP may also influence the SNS. High infusion rates of ANP have been shown to increase the release rate of catecholamines (Goetz, 1988). These data imply that the more prominent hypotensive effects of high doses of ANP elicit a more powerful reflex stimulation of the sympathetic nervous system by the arterial baroreceptors. The baroreceptor reflexes (probably) are responsible for the increases in circulating NE that have been detected after intravenous infusion of ANP in human subjects, and for the tachycardia that commonly occurs in human subjects in response to ANP (Goetz, 1988).

Another possible stimulus for ANP release during and following exercise may be elevated circulating pressor hormones such as NE and ANG II. Uehlinger et al. (1986) studied the release of endogenous ANP during infusions of NE and ANG II in 14 normal subjects. Individual doses of NE and ANG II were titrated so that mean blood pressure increased 20 mmHg. Plasma ANP increased significantly after 20 minutes of either NE or ANG II infusion, but the rise in ANP was more pronounced during NE (from 25 to 80 pg/ml) than during ANG II infusion (from 21 to 31 pg/ml). The depressor effect of ANP was also studied by infusing synthetic ANP. The depressor effect of ANP was more pronounced during NE infusion than during ANG II infusion. ANP

injected in increasing doses of 10, 40 and 75 micrograms progressively decreased blood pressure from 147/91 to 136/74 and 136/70 mmHg, respectively, during maintenance of NE infusion at the highest level.

ANP Release In HTR. It is now widely recognized that the release of ANP by atrial stretch represents a physiologic mechanism involved in maintenance of homeostasis in response to volume expansion. Cardiac innervation is not necessary for ANP release which has been found to occur in vitro from atrial fragments and in vivo in dogs with surgically denervated hearts. More importantly, because increased plasma ANP levels have been reported in HTR, the question may be asked whether Tx increases ANP secretion. However, factors other than interruption of cardiac nervous pathways may be implicated in increased plasma levels of ANP in HTR, such as 1) the increased atrial dimensions and myocardial mass due to the persistence of a cardiac remnant containing a rim of the native atria, 2) the renal insufficiency related to cyclosporine treatment, and 3) possible changes in atrial function.

The human HTR presents interesting ANP characteristics. Before Tx, several of the neuroendocrine systems involved in electrolyte balance and blood pressure regulation, RAAS and ANP in particular, are stimulated in the patient with severe CHF (Goldsmith et al., 1983a; Raine et al., 1986; Colucci et al., 1989; Francis et al., 1990; Swedberg et al., 1990). Long term follow-up of HTR has revealed persistent high levels of ANP release despite resumption of satisfactory hemodynamic status (Magovern et al., 1987; Singer et al., 1990; Dussaule et al., 1990; Mertes et al., 1991). At the same time, basal RAAS activity returns to nearly normal levels in most patients.

The mechanism underlying the raised ANP values in HTR is not clear. It has been suggested that elevated atrial pressure, ie. atrial stretch, due to central hypervolemia and/or hypertension may be the stimulus for higher ANP release (de Bold et al., 1981; Raine et al., 1986). There is evidence to indicate, however, that other factors may be responsible for the abnormally high ANP levels in HTR. Magovern et al. (1987) measured ANP in 19 HTR 1 year after Tx during routine endomyocardial biopsy. Plasma ANP levels in HTR were elevated 10 fold compared to normal (217 vs 20 pg/ml). Furthermore, ANP concentrations in HTR were 3 fold greater than a group of 20 coronary artery disease patients studied in the same laboratory during cardiac catheterization (217 vs 71 pg/ml). Despite the significantly higher ANP levels in HTR, there were no significant differences between the groups in right atrial pressure, pulmonary artery pressure, pulmonary wedge pressure or cardiac index. Studies that have measured plasma ANP in normal subjects during exercise have reported that high ANP levels persist up to 30 minutes during recovery from exercise despite the fact that atrial pressure drops rapidly (Donckier et al., 1988). The elimination half-life of ANP, ranging from 2 to 3 minutes, is too short to explain the sustained elevation of ANP.

Deray and coworkers (1988) measured plasma ANP in 3 HTR 1, 3 and 5 days after Tx. In all HTR, ANP had increased 20 to 74% from preTx levels despite reductions in right atrial pressure. No correlation was found between plasma ANP concentrations and right atrial pressure. The available data suggest that factors other than atrial filling pressure are important in determining ANP levels in HTR.

One possible explanation for the elevated ANP observed in HTR is systemic hypertension. Hypertension has been associated with modest elevations in ANP, but it has not been associated with the large magnitude of ANP increase that was found in HTR (Arendt et al., 1986). Others have speculated that the increased ANP levels in HTR are a response to the lower hemoglobin concentration (Magovern et al., 1987) owing to the fact that one of the primary actions of ANP is to cause hemoconcentration by shifting fluid out of the vascular space (Weidmann et al., 1986). Experimental evidence, however, is lacking to confirm this hypothesis.

Sympathetic innervation of the heart appears to be unnecessary for ANP to be released from atrial myocytes. Elevated basal levels of ANP have been reported in HTR early and late after Tx (Magovern et al., 1987; Deray et al., 1988; Mertes et al., 1991). Mertes and coworkers (1991) studied the short-term effects of Tx on the release of ANP during 10 days of recovery after Tx in a series of 10 HTR. PreTx ANP levels were elevated (42.4 pg/ml) but ANP dropped during day 1 of recovery (25.1 pg/ml). By day 10 of recovery, plasma ANP returned to elevated levels (38.4 pg/ml) that were comparable with those found before Tx.

Singer and coworkers (1990) measured ANP in 7 male HTR (47 yrs of age; 11-52 wks postTx) at rest and during a SL-GXT on an upright cycle ergometer. Resting ANP was significantly higher in HTR compared to a control group matched by age and gender (31.3 vs 8.0 pg/ml). ANP increased at a lower level of exercise in HTR but the maximal percentage increase in plasma ANP was not significantly different between groups (204 vs 151% for HTR and control groups, respectively).

ANP Release During Exercise. Plasma ANP has been shown to increase during exercise in normal subjects and the degree of increase is proportional to the relative intensity of the exercise (Donckier et al., 1988). Donckier et al. (1988) simultaneously measured plasma ANP, ventricular volumes and ejection fraction in 13 normal subjects (7 males; 6 females) during 5 minute bouts of recumbant cycle ergometry at 20, 40 and 60% of peak power output. Although baseline and exercise ANP levels were greater in women, the sex by time interaction was not significant. Both groups significantly increased plasma ANP (2 to 4 fold) at 40 and 60% of peak power output but not during exercise at 20%. The highest ANP concentrations were found at the same levels as the lowest ventricular volumes. The degree of atrial stretch associated with decreased ventricular volume was not measured during exercise.

Renin-Angiotensin-Aldosterone-System

The RAAS is an important endocrine system in electrolyte balance and cardiovascular hemodynamics. In this system, the enzyme renin secreted by the kidney into the circulation acts on the substrate angiotensinogen secreted from the liver to produce angiotensin I. This decapeptide is then converted by angiotensin-converting enzyme (ACE), a dipeptidyl peptidase found in large concentrations in the lungs, to the peptide hormone angiotensin II (ANG II). ANG II has multiple target organs (such as blood vessel, kidney, adrenal, heart, brain, pituitary, reproductive organs etc.) from which it can elicit specific responses (Guyton, 1986). The best-known functions of ANG II are its contributions to cardiovascular and renal homeostasis. ANG II is a potent vasoconstrictor in acute blood pressure homeostasis and also mediates

synthesis and release of aldosterone by the adrenal cortex in chronic blood pressure homeostasis.

Previous *in vitro* experiments have shown that cyclosporine stimulated renin production in rat kidney cortex slices by 43% (Bellet et al., 1985). Cyclosporine doses of 20 to 25 mg/kg daily have also been shown to stimulate plasma renin activity (PRA) levels *in vivo* with dogs and rats after several days. These results therefore suggested that cyclosporine affects the RAAS and consequently raises blood pressure. Bellet et al. (1985), however, reported that cyclosporine did not stimulate the RAAS in a group of 15 hypertensive HTR. Reported levels of plasma aldosterone, angiotensinogen and ACE activity were all normal. Furthermore, plasma renin activity and aldosterone were within normal limits after acute treatment with captopril (1 mg/kg body weight). However, they did find that plasma volume was increased by 15% as measured by the radioiodine-labeled serum albumin method (Bellet et al., 1985). The authors conclude that although stimulation of the RAAS is improbable in HTR, poor adaptation of the RAAS to fluid retention (15% increase) may be partly responsible for the increase in blood pressure.

Thames et al. (1971) also found that dogs with autotransplanted hearts had abnormally expanded blood volumes and subnormal increases in plasma renin response to hemorrhage. The authors conclude that the most likely explanation for the abnormal blood volumes and the abnormal renin responses to hemorrhage was that Tx interrupts reflexes from the heart which contribute to the regulation of blood volume. They speculate that it was unlikely that expanded blood volume was due to more severe hemodynamic disturbances since the changes in mean arterial blood

pressure and in mean right atrial pressure in the dogs were comparable to the normally innervated control dogs.

It is likely that the expanded blood volumes and the subnormal renin responses to bleeding in Thames' dogs after Tx are related in a cause and effect relationship. There are two possibilities: blood volume expansion might result from Tx in a manner which does not involve the mediation of the RAAS. The alternative possibility is that cardiac afferent denervation associated with Tx causes an increase in PRA by reflex mechanism and that this in turn results in expansion of blood volume.

RAAS in HTR. Held et al. (1989) studied the PRA response to sympathetic stimulation produced by infusion of isoprenaline and during change in posture in HTR (n=9) undergoing long-term treatment (6 to 12 months postTx) with cyclosporine. In normal subjects, an isoprenaline infusion is associated with a decrease in diastolic blood pressure and an increase in PRA. Despite similar decreases in diastolic blood pressure in both HTR and control groups (-17 vs -15 mmHg), PRA was reported to be significantly lower among HTR when compared with controls at baseline (0.7 vs 1.4 ng/kg/min) and did not increase during isoprenaline infusion (0.5 vs 2.5 ng/kg/ml) or after change of posture. The authors interpret their findings as an indication of a defect in the ability of the kidneys to release or produce renin in response to sympathetic (beta-receptor) stimulation. They conclude that the most probable explanation is a cyclosporine mediated toxic effect on the kidney.

Myers et al. (1988) studied the role of cardiac atria in the renal response to sequential volume expansion and contraction, during and directly following water immersion in 9 HTR. Water immersion is a maneuver

that leads to plasma volume expansion, atrial distension and release of ANP. An acute increase in plasma volume leads to immediate renal vasodilation, diuresis and natriuresis. The renal response is mediated in part by the release of hormones that act to directly relax renal vascular smooth muscle and inhibit tubular transport of sodium and water, and in part by the simultaneous inhibition of hormones and renal SNS traffic that causes vasoconstriction, antidiuresis and sodium retention. Nerve fibers in the walls of the atria that behave as mechanoreceptors play an important role in the inhibition of hormones and SNS outflow to the renal vasculature. On sensing an increase in either mechanical stretch or transmural pressure, they evoke signals that travel via the vagus nerve to integrative centers in the hypothalamus and medulla oblongata. These centers, in turn, exert a tonic inhibitory effect on renal vascular resistance and to promote natriuresis and diuresis by reducing SNS outflow to the renal vasculature. The authors noted that the most striking difference between control and HTR during and after water immersion was elevated vascular resistance and PRA in HTR. They conclude that the likely explanation for the elevated renal vascular resistance and PRA is unopposed and/or enhanced SNS traffic to the renal vasculature.

Pulmonary Function

Normal ventilation (Sietsema et al., 1987; Banner et al., 1988) and decreased ventilation (Marconi et al., 1987; Meyer et al., 1989) have been reported in HTR during the rest to exercise transition. Marconi and associates (1987) were the first group to suggest that tolerance for aerobic exercise in HTR may be limited by inadequate gas exchange kinetics rather

than by cardiac output abnormalities. Oxygen uptake ($T_{1/2} \dot{V}O_{2\text{-on}}$) during the rest to exercise transition was measured in 6 male HTR (1-8 mo post-op). $T_{1/2} \dot{V}O_{2\text{-on}}$ during cycle exercise at 85% of peak $\dot{V}O_2$ was slower for HTR compared to age-matched, untrained control subjects (65 vs 43 seconds).

Meyer et al. (1989) studied the kinetics of respiratory and cardiac function in 21 HTR and 10 healthy controls. Breath-by-breath analysis of respiration and impedance cardiography were used during 5 minutes of upright constant load cycle exercise at 50 watts. The half-time of the on-response for pulmonary ventilation was significantly slower (77 vs 42 seconds) in HTR but the kinetics of cardiac output (52 vs 40 seconds) were only moderately impaired. From these data, the authors concluded that respiratory gas exchange rather than cardiac function was the limiting factor during exercise of moderate intensity.

Contrastingly, Sietsema and coworkers (1987) reported that ventilation and $\dot{V}O_2$ increased promptly in 4 HTR during progressive and constant load cycle exercise. The kinetics of the increase in $\dot{V}O_2$ to its steady state were reported to be "within the range found in normals during moderate exercise" (42 ± 12 seconds).

Cardiac Denervation and DLCO

Diminished DLCO has been reported in HTR (Casan et al., 1987; Banner et al., 1988). Immunosuppression therapy with cyclosporine, the surgical procedure and reduced hemoglobin have been cited as possible mechanisms responsible for the diminished DLCO (Casan et al., 1987; Wright et al., 1990; Bussieres et al., 1990). However, the nature of the DLCO impairment in HTR is unknown and generalizations should not be made

because the available data does not include pre- and postTx pulmonary measures. Considering the fact that DLCO is markedly diminished in many CHF patients awaiting Tx (McNeil et al., 1958; Morley et al., 1989; Wright et al., 1990; Bussieres et al., 1990), it is possible that the abnormal postoperative DLCO observed in some HTR may not be a result of the surgical procedure or immunosuppression therapy but rather a persistence of preexisting conditions.

The data of Casan et al. (1987) showed a mean reduction of 14% in DLCO in 10 HTR 6 to 12 months after Tx despite significant improvements in FVC and FEV₁. The authors concluded that the reduction in DLCO was an early manifestation of cyclosporine induced lung toxicity similar to that which has been documented in the kidneys of renal transplant patients (Myers et al., 1984; Taube et al., 1985; Humes et al., 1985; Racusen et al., 1986; Macris et al., 1989). As indirect evidence for their conclusion, they reported a high correlation ($r=0.87$) between the reduction in DLCO and the level of cyclosporine in whole blood at the time of the pulmonary function tests.

Cyclosporine immunosuppression therapy is frequently complicated by the development of hypertension and early nephrotoxicity or hepatotoxicity (Myers et al., 1984; Taube et al., 1985; Humes et al., 1985; Racusen et al., 1986; Macris et al., 1989). Additionally, neurologic toxicity has been observed in liver transplant patients and HTR receiving cyclosporine (Berden et al., 1985; Adams et al., 1987; DeGroen et al., 1987; Vazquez de Prada et al., 1990). Several other side effects of cyclosporine have been reported as isolated case reports but none have been considered contraindications for cyclosporine therapy (Vazquez de Prada et al., 1990). Evidence for cyclosporine induced pulmonary dysfunction, however, has

not been presented in the related literature. Bennet and Norman (1986) in an earlier review make no reference to the adverse effects of cyclosporine on the lung. Copeland et al. (1986) describe only infrequent pleural edema secondary to cyclosporine therapy and these symptoms are transient. In a detailed review of drugs potentially toxic to the lung, Cooper et al. (1986) do not include cyclosporine. Macris et al. (1989) in a recent review of clinical contraindications for cyclosporine immunosuppression make no mention of pulmonary dysfunction.

Cardiac Denervation and Exercise Hyperpnea

Numerous hypotheses involving neural, humoral and combined neural-humoral mechanisms have been postulated for control of exercise hyperpnea and support for each has been found (Powers and Beadle, 1985). Most investigators agree that the initial fast phase (phase 1) of exercise hyperpnea is neurally mediated. However, the site of origin and magnitude of contribution remain contentious. The fast phase has been demonstrated to be mediated indirectly through feedback via afferent stimuli originating from skeletal muscle in the working limbs (Kao, 1963; Tibes, 1977) or directly via feedforward by efferent signals from higher centers of the brain (Eldridge et al., 1981).

Another neurogenic hypothesis to explain the immediate hyperpnea observed at the onset of exercise contends that the ventilatory response is dependent on the cardiac output response. Afferent signals from cardiac ventricular stretch receptors have been shown to stimulate ventilation in animals (Wasserman et al., 1974, Uchida, 1976; Jones et al., 1982). Jones et al. (1982) found that the relationship between minute ventilation and changing right ventricular load in anesthetized dogs was $r=0.94$ ($p \leq 0.01$).

Cardiac denervation, a necessary consequence of Tx, would be expected to ablate reflex stimuli originating from the heart thereby reducing the ability to appropriately control pulmonary ventilation. Denervation of all cardiac afferents would be expected to result in hypoventilation during the onset of exercise and be characterized by transient hypercapnia and hypoxemia.

Banner et al. (1988) investigated this hypothesis by examining the ventilatory and circulatory responses at the onset of exercise in 6 HTR, 5 subjects with combined heart-lung transplants and 7 normal controls. The subjects sat in a chair and pedalled a lever-arm system. Exercise consisted of a one minute push-relax rhythm produced either voluntarily by the subjects or involuntarily by electrical stimulation of peripheral nerves of the quadriceps and hamstring muscle groups. Ventilatory variables and cardiac output were measured as average values over 4 consecutive 30 second periods starting 1 minute before the onset of exercise. There were no significant differences between voluntary and involuntary initiation of exercise for any of the respiratory or cardiovascular variables. Oxygen consumption, carbon dioxide production and the ventilatory and respiratory rate responses were not significantly different between HTR and control subjects. Percentage changes in ventilation were plotted against percentage changes in cardiac output revealing that normal or even exaggerated ventilatory responses at the onset of exercise can occur without a proportional underlying rise in cardiac output in the heart-lung transplant group. The authors concluded that the ventilatory and circulatory responses are also dissociated at the onset of exercise in HTR.

Skeletal Muscle Strength

It has been suggested that skeletal muscle weakness may be a primary mechanism responsible for exercise intolerance in otherwise healthy HTR (Kavanagh et al., 1988). To date, however, no data exists comparing peripheral muscle strength in HTR with normative values. Furthermore, those studies that have conducted body composition analyses (ie., fat vs fat-free tissue) in HTR are sparse.

Preoperative physical activity is typically restricted in Tx candidates due to severely limited cardiac output. An extended waiting period for a donor organ to become available may result in considerable deconditioning and atrophy resulting from inactivity. Following Tx, chronic corticosteroid (eg. prednisone) therapy for immunosuppression is recommended for most HTR. Corticosteroids cause pronounced atrophy of skeletal muscle (steroid myopathy) when administered at high doses for prolonged periods of time (Goldberg and Goodman, 1969; Dujovne and Azarnoff, 1975; Kinney and Felig, 1979). Steroid induced myopathy leads to reduction in muscle mass, depletion of muscle protein and loss of strength. An important aspect of steroid myopathy is that the skeletal muscle fiber-type response within the musculature is variable. Fast-twitch white or glycolytic fibers (Type II b) are significantly atrophied in response to chronic levels of corticosteroids, while slow-twitch oxidative fibers (Type I) are relatively resistant to atrophy (Goldberg and Goodman, 1969; Kelly and Goldspink, 1982; Seene and Viru, 1982; Almon and Dubois, 1990). An increasing body of evidence has shown that muscle atrophy from corticosteroids can be prevented or retarded by exercise training (Seene and Viru, 1982; Horber et al., 1985; Hickson et al., 1986; Czerwinski et al., 1987;

Horber et al., 1987) but no data are available concerning strength training results in HTR.

Weight gain after Tx is typical for most HTR (Keteyian et al., 1989) but little information is available to indicate whether the gain is fat or fat-free tissue. Keteyian et al. (1989) observed an average 11 kg increase in total bodyweight at 10 months after Tx in 47 HTR receiving triple immunosuppression therapy with cyclosporine, azathioprine and prednisone. A significant increase in bodyweight was found in all patients, independent of preTx weight and participation in a postTx 7- to 12-week exercise training program. The conclusions that can be drawn from these data, however, are limited by the fact that body composition analysis was not performed before or after the rehabilitation programs.

Kavanagh and coworkers (1988) report a 5.0 and 1.3 Kg increase in total bodyweight for moderately and high compliant HTR, respectively, participating in a 16 month walk/jog exercise program. Body composition analysis with skinfold measurements revealed that body fat had not increased significantly but lean mass increased an average of 2 Kg for all patients. The changes in body composition were accompanied by training induced increases in $\dot{V}O_{2\max}$ (mean 4.2 ml/kg/min), peak heart rate (mean 13 beats/min) and peak power output (mean 49 watts). Although there are several possible explanations for these responses, the authors speculate that the increase in performance could largely be attributed to strengthening of the leg muscles. The authors conclude that because changes in cardiac output were not observed over the period of training in HTR, the adaptations were peripheral rather than central and strengthening of skeletal muscles allowed the transplanted heart to "realize

its full physiologic and functional potential." Unfortunately, strength measurements were not included in their otherwise excellent exercise training study.

Horber and associates (1985, 1987) have investigated the impact of regular physical training on prednisone-induced myopathy in clinical populations. Compared with normal matched control subjects, 12 kidney transplant patients had a 20% lower mid-thigh muscle area, a 36% increase in mid-thigh fat-to-muscle ratio and a 20% lower peak torque output of the thigh musculature during isokinetic strength testing. After 50 days of isokinetic strength training, muscle area of the thigh increased, the fat-to-muscle ratio of the thigh decreased and mean peak torque reached normal values. These results have not been replicated in HTR.

CHAPTER 3 METHODS

Subjects

Eleven patients (n=10 males; n=1 female) who underwent Tx surgery at Shands Hospital, Gainesville, Florida, volunteered to participate in the study. Tx had been performed according to the techniques described by Baumgartner et al. (1990). The original indications for Tx were idiopathic cardiomyopathy (7 patients) and ischemic cardiomyopathy (3 patients) while one patient was a retransplant. The patients averaged 50.1 ± 13.7 (mean $\pm$ SD) years of age (range=21-63 years) and they were studied 18.7 ± 11.7 (mean $\pm$ SD) months after cardiac Tx (range=7-41 months).

Preliminary screening of the HTR was performed by clinical personnel from the Shands Hospital Heart Transplant Team. Contraindications to participate in the study included 1) a history of coronary artery disease since Tx; 2) biopsy or physical examination evidence of donor organ rejection requiring therapy since Tx; 3) supplemental oxygen therapy; 4) evidence of significant renal dysfunction; and, 5) significant cardiac, pulmonary or orthopedic complications during graded exercise testing. Those HTR that appeared suitable for the study were invited to the Center For Exercise Science for further screening. All HTR were medically stable and had no clinical or endomyocardial biopsy findings of significant rejection, infection or other major illness. Nine HTR were receiving triple-drug immunosuppressive therapy with

cyclosporine, prednisone and azathioprine while two HTR were treated with cyclosporine and prednisone. Four of the HTR were receiving non-thiazide "loop" diuretics (furosemide) for fluid retention and all were receiving antihypertensive drugs for mild-to-moderate hypertension. Antihypertensives included clonidine (3 pts), nifedipine (6 pts), captopril (3 pts) and enalapril (8 pts). No beta-blockers or other cardiac medications were used by HTR at the time of the study. It was deemed unsafe to withhold antihypertensive medications in certain HTR, therefore, all HTR were instructed to comply with their usual protocol of daily medications during the study and on the days of experiments.

The control group consisted of 11 subjects that were selected to match the HTR, as closely as possible, with respect to age, gender and body composition. They were normally active but not athletic and had no evidence of cardiac or pulmonary disease as determined by clinical examination, pulmonary screening and graded exercise testing. None of the control subjects were receiving any medication at the time of the study.

All subjects were required to report to the Center For Exercise Science for experimental testing on two days separated by a minimum of 72 hours. The series of tests on the first day habituated subjects to the laboratory procedures, measured body composition, pulmonary function and peak heart rate, power output, systemic oxygen consumption, neuroendocrine activity and provided exercise intensity criteria to be used during the second day of tests. On the second day bilateral knee extension strength and cardiac output, arterial blood gas and neuroendocrine activity were measured at rest and during cycle exercise at approximately one- and two-

thirds of the previously determined peak power output. All subjects were asked to restrict strenuous physical activity for 24 hours before experiments and report to the laboratory 2-3 hours postprandial.

Day 1: Experimental Protocol

Orientation

Upon arriving at the Center For Exercise Science, the entire research protocol and the inherent risks were outlined to the subject. The protocol was approved by the Institutional Review Board for the protection of human subjects at the University of Florida College of Medicine. All subjects provided their written informed consent (Appendix) to participate in the study. The subjects then completed questionnaires that dealt with demographic information, cardiovascular health, physical activity status and smoking history.

Body Composition Analysis

Body composition was then assessed anthropometrically from the sum of seven skinfold sites (chest, axilla, triceps, subscapula, abdomen, suprailium and thigh) measured on the right side of the body with a Lange skinfold caliper (Cambridge Scientific Industries, Cambridge, Md.). The methods used for taking skinfolds have been outlined by Pollock and Wilmore (1990). Subcutaneous fat was measured by grasping a skinfold of fat firmly by the thumb and index finger. The caliper was held perpendicular to the fold at approximately 1 cm from the thumb and forefinger. The caliper grip was then released so that the full tension was exerted on the skinfold. Body density (D_b) was predicted for males and females respectively with the following equations: males: $D_b = 1.11200000 -$

0.00043499 (sum of 7)+0.00000055(sum of 7)²-0.00028826(age)(Jackson and Pollock, 1978); and females: $D_b = 1.0970 - 0.00046971(\text{sum of } 7) + 0.00000056(\text{sum of } 7)^2 - 0.00012828(\text{age})$ (Jackson et al., 1980). The Siri equation (Siri, 1961) was then used to estimate percent fat: where $\%fat = ((4.950/D_b) - 4.50) \times 100$. Height was measured to the nearest 0.1 cm on a wall-mounted Harpenden stadiometer (Psister Import, #98-602, Carlstadt, NJ) and weight to the nearest 0.1 Kg on an Acme Medical Chair Scale (model 4000-0030, San Leandro, Ca.).

Pulmonary Function Tests

Pulmonary function tests were performed before and after Tx. Before Tx, the pulmonary tests were performed in the Lung Station at Shands Hospital as part of the routine preTx evaluation using a Collins Pulmonary Module (model Apex DS/620, Braintree, Ma). All pulmonary function tests completed after Tx were performed in the Center For Exercise Science using a Medical Graphics Corporation pulmonary testing unit (model 1070, St. Paul, Mn.). The unit consisted of a pneumotachometer, analyzers, computers and software necessary in the measurement of pulmonary spirometry, lung volumes and diffusion capacity. Pulmonary measurements were compared with reference values derived from prediction equations based on individuals randomly selected from the general population (Crapo et al., 1981; Miller et al., 1983). All pulmonary function test values before and after Tx were calculated using the same gender specific prediction equations with appropriate corrections for age, height, race and smoking history.

Tests were performed with the subject seated and wearing a noseclip. The mouthpiece was positioned so that the chin was slightly elevated and

the neck extended. The investigator demonstrated the proper technique before all maneuvers and the subject performed a practice test. Repeated trials of all tests were performed until two trials were in close agreement. The means of these two trials served as the criterion value. Verbal encouragement was given to help the subjects perform the procedures properly.

Pulmonary spirometry. A flow-volume loop test was used to generate values for forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁). The flow-volume loop test involved having the subject inspire maximally and then exhale forcefully to residual volume followed by a maximal inspiration. The FVC is the largest volume of gas measured on complete expiration after the deepest inspiration. The reference FVC values for males and females respectively were calculated with the following equations (Crapo et al., 1981): males: $FVC = (0.06000 \times \text{height}) - (0.0214 \times \text{age}) - 4.650$; and females: $FVC = (0.0491 \times \text{height}) - (0.0216 \times \text{age}) - 3.590$. The FEV₁ is the volume of gas expired over a one second interval during the performance of a FVC. The reference FEV₁ values for males and females respectively were calculated with the following equations (Crapo et al., 1981): males: $FEV_1 = (0.0414 \times \text{height}) - (0.0244 \times \text{age}) - 2.190$; and females: $FEV_1 = (0.0342 \times \text{height}) - (0.0255 \times \text{age}) - 1.578$.

Pulmonary diffusion capacity. The single-breath technique was the method of choice used to measure pulmonary diffusion capacity for carbon monoxide (DLCO). DLCO measures all the factors that affect the diffusion of a gas across the alveolar-capillary membrane. DLCO was

measured with appropriate correction for the subject's hemoglobin level on the day of the pulmonary function tests (Cotes and Hall, 1970).

After exhaling maximally the subject rapidly filled their lungs with a 4-gas diffusion mixture containing 0.5% neon (Ne), 0.3% carbon monoxide (CO), 21% oxygen (O₂) and 78.2% nitrogen (N₂). The subjects held their breath for 9-10 seconds and then exhaled completely. A minimum of 7 minutes was allowed between DLCO trials to insure adequate washout of the diffusion gas mixture. The DLCO was recorded in milliliters of CO diffused per minute per millimeter of mercury. The milliliters of CO transferred is the difference in concentrations of CO in alveolar gas at the beginning and end of the 10-second breath holding interval. DLCO was calculated according to the procedure described by Ruppel (1975). The reference DLCO_{sb} values for nonsmoking males and females respectively were calculated with the following equations (Miller et al., 1983): males: $DLCO_{sb} = 12.9113 - (0.229 \times \text{age}) + (0.418 \times \text{height})$; and females: $DLCO_{sb} = 2.2382 - (0.1111 \times \text{age}) + (0.4068 \times \text{height})$.

Alveolar volume (V_A) was also estimated during the diffusion test by using inert and insoluble Ne in the diffusion mix. V_A values were considered estimates of total lung capacity (TLC_{sb}) and they were subsequently used in all calculations requiring total lung capacity values. The reference TLC_{sb} values for males and females respectively were calculated with the following equations (Miller et al., 1983): males: $TLC_{sb} = (0.1930 \times \text{height}) - (6.6896)$; and females: $TLC_{sb} = (0.1641 \times \text{height}) - (5.4404)$.

Pulmonary diffusion capacity per unit of lung volume was calculated by dividing total pulmonary diffusion by total lung volume (DLCO/TLC). The reference DLCO/TLC values for nonsmoking males and females,

respectively were calculated with the following equations (Miller et al., 1983): males: $DLCO_{sb}/TLC_{sb} = (10.0882 - (0.0570 \times \text{height}) - (0.0309 \times \text{age}))$; and females: $DLCO_{sb}/TLC_{sb} = (8.3297 - (0.0460 \times \text{height}) - (0.0157 \times \text{age}))$.

Symptom Limited- Graded Exercise Test

Before the symptom limited-graded exercise test (SL-GXT), each subject underwent a physical and cardiovascular examination administered by a physician. Those subjects deemed suitable were then prepared to undergo a SL-GXT on a Combi System 5HR electromagnetically braked cycle ergometer (Combi/Mitsui Co., Cleveland, OH). All SL-GXT's were supervised by a physician trained in graded exercise testing. A crash cart with all necessary emergency medications, supplemental oxygen and a defibrillator was immediately adjacent to the cycle ergometer during every SL-GXT.

A short 20 or 22 gauge polyethylene cannula was inserted into an antecubital vein under aseptic conditions and kept open by filling the catheter with dilute heparinized saline. After a 30 minute seated rest, 15 milliliters of venous blood was drawn into a single plastic syringe and immediately separated into individual aliquots for norepinephrine (NE), arginine vasopressin (AVP), plasma renin activity (PRA), atrial natriuretic peptide (ANP), angiotensin II (ANG II) and aldosterone (ALDO) assays (see section entitled, "Blood Sample Collection").

Subjects then rested supine while blood pressure was measured by auscultation using a standard mercury sphygmomanometer. Heart rate and a 12-lead electrocardiogram (ECG) were recorded with standard limb lead placements using a Quinton Q 4000 system (Quinton Instruments, Seattle, Wa.) with a three channel oscilloscope for ECG display. ECG limb

lead placements were then moved to abbreviated positions on the torso and subjects returned to a seated position where blood pressure and a 12-lead ECG were again recorded before and after 30 seconds of hyperventilation. Measurements of heart rate and blood pressure with the subject seated prior to mounting the cycle ergometer were considered criterion baseline values. Subjects were then seated on the cycle and the seat height was adjusted so that the legs were in a position of slight flexion at the nadir of the downstroke. After a 5 minute rest, blood pressure and a 12-lead ECG were again recorded. If blood pressure exceeded 180/100 mmHg or if any significant ECG abnormalities were evident during the series of measurements made at rest such as life-threatening arrhythmias, ST segment depression that was 1mm horizontal or downsloping, or 2mm upsloping 0.08 seconds from the J-point, ST segment elevation, or any other ECG patterns associated with significant cardiovascular disease, subjects were excluded from the study (see Appendix A, "Criteria for Exclusion of Subjects").

The SL-GXT was a continuous, multistage protocol with an increase in resistance every minute until the subject reached voluntary maximal exertion or became symptomatic with positive hemodynamic or medical indices. The test began with 5 minutes of quiet rest while the subject sat on the cycle. The transition from rest to the predetermined initial exercise stage was slow, with intensity increasing in a ramp-like manner during a period of 1 minute. The initial exercise power output was 20 watts for the HTR and 40 watts for the controls. During the rest to exercise transition and throughout the test subjects pedalled at 60 revolutions·min⁻¹ (rpm). After one minute at the initial exercise stage, power output was increased

every minute by 10 watts for the HTR and 20 watts for the control subjects in an attempt to bring about exhaustion within an 8-12 minute period. A 12-lead ECG and rating of perceived exertion (Borg, 1962) was recorded at the end of each one-minute interval. Blood pressure was measured during the last 20 seconds at 2 minute intervals with the subjects arm supported at chest level.

One minute collections of expired air were taken in neoprene meteorological balloons during the final 3-4 minutes of exercise for the measurement of peak systemic oxygen consumption ($\dot{V}O_2$), expired carbon dioxide ($\dot{V}CO_2$) and expired pulmonary ventilation ($\dot{V}_E$). Samples of the expired gasses were analyzed by a mass spectrometer (Perkin-Elmer, MGA model 1100) for fractional concentrations of oxygen and carbon dioxide. The mass spectrometer was calibrated with dry gas samples of known concentrations. Expired volume was measured with a 120 L Tissot chain-compensated spirometer (Collins) and corrected to STPD conditions for $\dot{V}O_2$ calculations as described previously by Consolazio et al. (1963).

Subjects were verbally encouraged to continue exercise as long as possible. The test was continued until a pedal frequency of 60 rpm could not be maintained. Immediately upon completion of the test another 15 milliliter venous blood sample was drawn from the indwelling catheter. During the first two minutes of recovery, subjects continued to pedal at a rate of 40-50 rpm with zero resistance. After two minutes of active recovery on the cycle, the subjects were seated in a chair and heart rate and blood pressure were recorded for an additional 8 minutes. Several HTR were monitored beyond 10 minutes while the exercise-induced tachycardia gradually diminished to baseline heart rate levels.

Day 2: Experimental Protocol

Subjects reported to the Center For Exercise Science for the second series of experiments a minimum of three days after Day 1. Day 2 exercise tests were performed at approximately the same time of day (12:00-3:00 pm) as the SL-GXT on Day 1 in order to control for a possible effect of circadian rhythms. Environmental conditions within the laboratory were maintained relatively constant at 22-23°C, 56-62% relative humidity and 760-765 mmHg barometric pressure during the course of all experiments.

To determine the temporal pattern of cardiac hemodynamics, ABGs and neuroendocrine activity during exercise, each subject was studied at two levels of exercise intensity on the same electronically braked cycle ergometer (Combi 5RH) used during the SL-GXT. Cardiac output, ABG and neuroendocrine measurements were made at rest and during two 10 minute periods of constant load cycle exercise. The two submaximal exercise tests were separated by 20 minutes of seated rest. The relative exercise intensities selected for the study were 1) 40% and 2) 70% of the peak power output (Watts) achieved during the SL-GXT on Day 1. These power outputs were chosen because they represent light and vigorous exercise intensities, respectively. It was felt that 40% of peak power output would increase neuroendocrine activity moderately and 70% was expected to increase neuroendocrine activity to a significantly higher level. Using heart rate to establish exercise intensity was considered inappropriate in HTR. The linear relationship between heart rate and $\dot{V}O_2$ cannot be used in HTR because this method does not reflect the correct exercise intensity in this population (Keteyian et al. 1989). Moreover, it seemed inappropriate to use $\dot{V}O_2$ to establish relative exercise intensity. Previous

experiments (Barr et al., 1964) have shown that subject apprehension often alters the normal breathing pattern and thus may influence the behavior of ABG. It was felt that the novelty of using the breathing valve and attached headgear as well as the elevated resistance to airflow introduced by the rebreathing valve might alter the respiratory rhythm and ABG measurements.

Subject Preparation

Each subject was prepared with a radial artery catheter in the nondominant arm by a physician trained in the technique. The dependent limb was examined by the Allen Test prior to catheter insertion to insure adequate collateral (ulnar artery) circulation to the hand and fingers. After aseptic preparation of the skin with Betadine swabs, an injection of 1% Xylocaine was administered for local anesthesia and a 20-gauge arterial cannula was placed percutaneously in the radial artery. A three-way stopcock and 3 inch t-connector were fastened to the catheter and kept patent with dilute heparinized saline. This instrumentation permitted frequent arterial blood sampling to determine the temporal pattern of ABGs and plasma levels of NE, AVP, PRA, ANP, ANG II and ALDO during exercise. A physician was always present to direct their attention exclusively to the blood sampling and the condition of the subject.

After placement of the arterial catheter, subjects remained seated for 20 minutes while four Mylar strip electrodes (3M Co., St. Paul, Mn.) were applied for use in the noninvasive measurement of cardiac output. Two electrodes encircled the neck and two electrodes encircled the thorax with one at the level of the xiphisternal joint and the other approximately 5 cm

lower. Cardiac output data were obtained from the electrodes using a Minnesota Impedance Cardiograph and Surcom Cardiographic microcomputer (model 7000, MPLS, Mn,) as first described by Kubicek et al. (1966).

Submaximal Cycle Test

Each submaximal exercise test was preceded by 10 minutes of quiet rest with the subject seated on the cycle. After the rest period 4-6 Impedance Cardiography measurements were made and blood samples were drawn for baseline ABG, hematocrit (Hct), hemoglobin (Hb) and neuroendocrine hormone analysis. Thus, there was a total of 30 minutes between catheter insertion and withdrawal of the first blood samples. After collection of the final resting blood sample, the subject began pedalling at 60 revolutions per minute with the desired resistance preselected, i.e. 40% of peak power output (Watts). Impedance Cardiography measurements (in triplicate) and blood samples for ABG analysis (1.5 ml) were taken every 30 seconds during the first 5 minutes of constant load exercise and at minute 6, 8 and 10 during the second 5 minutes of the exercise bout. Blood samples for neuroendocrine hormone analysis (8 ml) were drawn at minutes 2, 4, 6, 8, and 10 during exercise. The blood sampling periods were chosen to allow detection of any possible ABG or plasma neuroendocrine hormone alterations that might occur during the transition from rest to exercise and during sustained constant load exercise. A small portion of blood (< 1ml) was also drawn before and immediately after each 10 minute ride for measurement of Hct and Hb. At the end of the first 10 minute cycle test at 40% of peak power output, the subject stopped pedalling and rested quietly in a chair for 10 minutes. The

second cycle test at 70% of peak power output also began with the subject seated quietly for 10 minutes on the cycle, thereby providing a total of 20 minutes of seated recovery between tests. The same pattern of data collection as described above was repeated during the second 10 minute ride at 70% of peak power output.

Cardiac output. Cardiac output was non-invasively estimated at rest and during submaximal exercise with the Impedance Cardiograph. This tonometric technique evaluates changes in the electrical conductivity of the thorax that result from pulsatile changes in thoracic blood volume caused by velocity and volumetric variations in aortic blood flow during the cardiac cycle. Measured changes in impedance (Ohms) during systole are used to estimate stroke volume and calculate cardiac output (Kubicek,1966). An imperceptible constant sinusoidal current of 4 milliamperes with a frequency of 100 kilohertz is introduced through the outer two electrodes. The two inner electrodes detect impedance or voltage changes during the cardiac cycle. The impedance signal is modified by variations in the velocity and volume of aortic blood during the cardiac cycle. The average of 4-6 cardiac cycles was used to estimate cardiac output at rest and 2-3 cardiac cycles were averaged for each cardiac output estimate during exercise.

Maximal Strength Testing

After the submaximal cycle ergometer experiments were completed, the arterial catheter was removed and the subject rested in a chair for approximately one hour. Each subject then completed a dynamic bilateral knee extension strength test on a Nautilus TM (Dallas, Tx) variable resistance knee extension tensiometer. Maximal knee extension strength

was defined as the maximum weight that could be lifted for one repetition of the movement (1-RM). Subjects were positioned in the tensiometer chair and the back rest was adjusted so that the axis of rotation about the knee extended approximately 5 cm beyond the edge of the chair. The angle of the back rest was fixed at 110° with respect to the seat. Subjects were secured in place with a seat belt placed around the pelvis. During each contraction, subjects were required to maintain contact with the back rest and to hold onto hand grips located on each side of the seat.

Each testing session began with the subject performing a warm-up set of 6-8 repetitions with a light weight. Two minutes of rest were given between the warm-up and the start of the 1-RM test. The initial 1-RM was standardized among subjects and represented approximately one-third of their body weight. When the weight was successfully lifted through a 120° range of motion (ROM= 60° to 180° of knee extension) the weight for the next trial was incremented by 2.3 to 9 kg. A 1-RM trial was considered successful if the weight was lifted within 10° of full extension to allow for compression of pads and leg tissue. The increments in weight were dependent upon the effort required for the lift and became progressively smaller as the subject approached the 1-RM. Two minutes of recovery time were allowed between 1-RM attempts. The last weight successfully lifted through the full ROM was considered the 1-RM. Most subjects reached their 1-RM in 3 to 5 trials.

Blood Sample Collection

Blood was obtained from an indwelling plastic catheter before and immediately after the SL-GXT in Day 1 and before and during each 10 minute submaximal constant load cycle ride in Day 2. A total of 30 ml of

venous blood was obtained in a SL-GXT and 75-80 ml of arterial blood was drawn in a typical submaximal 10 minute cycle test and recovery period for a total of 150-160 ml of arterial blood on Day 2. Blood samples were drawn after aspirating and discarding the contents of the catheter and T-connector dead space (~1 ml). Each ABG sample was uniformly drawn under anaerobic conditions using a small (3 ml) heparinized plastic syringe. After the ABG sample was drawn, the syringe was immediately capped and stored on ice until analysis. Immediately before and after each exercise test a small portion of the blood sample was placed in 75mm coated glass capillary tubes (duplicates) and centrifuged for measurement of Hct and Hb. Blood samples for neuroendocrine hormone analysis were withdrawn into a plastic syringe containing no additives and immediately separated into individual aliquots. Blood for NE assay (2 ml) was added to chilled heparinized vacutainers containing 40 microliters of antioxidant consisting of ethylene glycol-tetraacetic acid (EGTA, 90mg/ml) and reduced glutathione (75mg/ml)(Sigma Chemical). Blood for AVP, PRA, ANG II and ANP assay (6 ml) was added to a chilled vacutainer containing ethylene diamine-tetraacetic acid (EDTA). Blood for ALDO assay (4 ml) was added to a vacutainer containing no additives and left at room temperature for 20 minutes. The blood samples were immediately mixed and plasma or serum was separated by centrifugation at $2,200 \times g$ at 4°C for 20 minutes. The plasma or serum was then pipetted to polypropylene tubes which were frozen at either -40°C (AVP, PRA, ANP) or -80°C (NE, ANG II, ALDO) until the end of the study so that all samples for each subject could be run in the same assay.

Blood Sample Analysis

Blood gas analysis. ABG and pH analysis were performed on a Nova Stat 5 ABG and acid-base analyzer (Nova Biomedical, Waltham, Mass.). The instrument autocalibrated using precision gasses and buffers after each sample was analyzed. ABG samples were kept on ice until analysis and determinations were performed within 60 minutes after sample collection.

Vasopressin radioimmunoassay. Plasma AVP was measured by radioimmunoassay using a rabbit anti-AVP antibody. The anti-AVP serum was generated in the laboratory of Dr. C. Wood, Department of Physiology, University of Florida, using AVP (Sigma Chemical) covalently linked to bovine thyroglobulin with carbodiimide. The crossreactivity of the antiserum with lysine vasopressin was 0.7%. Crossreactivity with oxytocin, vasotocin, AVP (fragment 4-9), CRF, ACTH, angiotensin I, and angiotensin II was each <0.001%. AVP was extracted from plasma by adsorption to bentonite. Plasma (1.0 ml) was extracted and then reconstituted to 0.25 ml with assay buffer (0.05 M phosphate buffer, pH=7.4 containing 0.01 M EDTA and 0.2% BSA). ^{125}I -AVP (Dupont C., Wilmington, DE.) was used as tracer. The range of the standard curve was from 0.05 to 10 pg/tube. In previous studies using this assay, the intraassay coefficient of variation (CV) for a low pool (0.40 pg/tube) was 4% and for a high pool (4.0 pg/tube) was 14%. Interassay CV was 7% (0.35 pg/tube).

Plasma renin activity radioimmunoassay. PRA was measured using a modification of the method of Haber et al. (1969). Angiotensin I (ANG I) was generated at pH 5.7 at both 37° and 0° C. Before generation of ANG I, plasma sample pH was adjusted by addition of 0.5 M citric acid plus 1 M

phosphate buffer (1:1.1 dilution of the sample) and protease activity was inhibited by addition of phenylmethylsulfonyl flouride (2% in ethanol, 1:1.01 dilution; Sigma Chemical). ANG I was generated for 1.5 hours. The generation was linear over the total time of the incubation. ANG I was measured by radioimmunoassay using rabbit anti-ANG I generated in the laboratory of Dr. I. Phillips, Department of Physiology, University of Florida. The range of the standard curve was from 0.078 to 5 ng/ml. PRA was expressed as the rate of generation of ANG I at 37° C minus the generation of ANG I at 0° C. In previous studies from this laboratory the interassay CV was 24.4% and the intraassay CV was 17.5%. The relation between the PRA results using the present assay and PRA results using a kit from Clinical Assays (Baxter Healthcare, Cambridge, Mass.) are as follows: Clinical Assays kit = (present assay) $\times$ (0.70 $\pm$ 0.06); $r^2 = 0.93$.

Atrial natriuretic peptide radioimmunoassay. ANP was extracted from thawed plasma samples using SepPak C₁₈ (octadecyl silica) cartridges (Waters Associates) which had been wetted and prewashed with 5 ml methanol, 5 ml 1% trifluoroacetic acid (TFA), 1 ml 1% polypep solution, 5 ml methanol/H₂O/TFA (80:19:1) and 5 ml 1% TFA. The samples were acidified with an equal volume of 1% TFA and applied to the cartridges. The cartridges were then washed with 5 ml 1% TFA/1% NaCL and the ANP was eluted with 3 ml methanol/H₂O/TFA (80:19:1). The eluate was dried down under air at 35° C.

The radioimmunoassay procedure was begun by reconstituting the ANP samples. A 0.1 M phosphate buffer with 0.05 M NaCL, 0.1% bovine serum albumin (BSA), 0.1% Triton X-100 and 0.01% NaN₃ was used to dissolve and dilute samples, standards, antibody and ¹²⁵I-labeled ANP

(rat) (New England Nuclear Research Products). The rabbit anti-ANP serum was generated in the laboratory of Dr. I. Phillips, Department of Physiology, University of Florida. Antibody was added to the standard or samples and incubated for 24 hours at +4° C. Iodinated ANP was then added and incubated an additional 24 hours at +4° C. Bound ANP was precipitated with goat-anti-rabbit antiserum and normal rabbit serum (both from Biotek Research) by a 2 hour incubation at room temperature. The samples were centrifuged at 2,000 g for 30 minutes at +4° C, the supernatant was discarded and the precipitate was counted in a gamma counter (model 5500, Beckman). Counts per minute from the gamma counter were fed directly into a DP5500 Beckman data reduction system program for logit-log method of analysis developed by Rodbard and Lewand (1970). This program calculates the best fit straight line by use of the linear regression statistical method. The recovery for ANP by the above extraction procedure was found to be $87\pm 6\%$ and the data presented in this study have not been corrected for the recoveries.

Aldosterone radioimmunoassay. Plasma ALDO concentration was measured by RIA in unextracted serum using a kit from Diagnostics Products Corp., Los Angeles, CA. Serum samples (0.200 ml) were added to polypropylene tubes coated with antibodies to ALDO. 1.0 ml of ^{125}I aldosterone tracer was added to the tubes and after a 3 hour incubation at 37°C the supernatant was decanted and the tubes counted on a gamma counter. ALDO concentration was determined from a standard curve. Intraassay CV for this procedure is 2.7-8.3% and interassay CV is 3.6-10.4%. The detection limit is approximately 16 pg/ml.

Angiotensin II radioimmunoassay. Plasma ANG II was measured by RIA after extraction on bentonite. This assay has been described more fully elsewhere (Ben et al., 1984; Raff et al., 1985). The antiserum used has less than 1.0% cross-reactivity with ANG I and was generated in the laboratory of Dr. C. Wood, Department of Physiology, University of Florida.

Norepinephrine. Plasma concentrations of NE were measured by high performance liquid chromatography (HPLC) (ESA Coulochem, Bedford, MA). NE was extracted by adsorbing plasma samples onto alumina. After washing the adsorbed alumina with buffer solution, NE was eluted from the alumina by treating it with an acid solution. 3, 4-Dihydroxybenzylamine (DHBA) was used as an internal standard and extraction efficiency of NE and DHBA was based on the extraction of known standards. All NE samples were corrected for percent recovery. After extraction, the NE samples were assayed by injecting the samples onto a reverse-phase C¹⁸ column. An ESA 16-channel Coulochem multi-electrode array detector was used to determine the concentration of NE in the samples. The within-assay CV was 1.2% and the between assay CV was 3.2%.

Cyclosporine radioimmunoassay. Cyclosporine concentrations were measured in whole blood specimens by Fluorescent Polarization using an Abbott TDX Fluorometer (Abbott Laboratories, Abbott Park, IL). The cyclosporine was labelled with fluorescein to obtain fluorescent properties and measured using a sheep anti-cyclosporine antibody (Abbott). The intraassay CV was 2.3% and the interassay CV was 6.1%. The average

recovery from samples was 95% and the samples were not corrected for recovery.

Hematocrit and Hemoglobin. Hct and Hb determinations were made with a QBC II Centrifugal Hematology system (Becton Dickinson, Franklin Lakes, N.J.). The QBC II method used fluorescence measurements of blood cells treated with fluorochrome acridine orange and the density gradient layering of blood cells to measure the volumes of red cells, white cells and platelets. Layer measurements were used to compute Hct. Hb concentration was derived from the Hct and measurements of red cell density as previously described by Wardlaw and Levine (1983). The percent change in plasma volume during each exercise test was calculated from the preexercise and postexercise Hct values (Van Beaumont et al. 1973).

Statistical Analysis

Descriptive characteristics, knee extension strength, pulmonary function and SL-GXT measures were compared between groups using analysis of variance (ANOVA). ANOVA was also used to compare pre- and postTx pulmonary function measures in the HTR.

Pearson product-moment correlation coefficients were calculated to determine the relationships among cyclosporine levels, smoking history and pulmonary diffusion capacity, and the changes in arterial partial pressure for oxygen during exercise.

Because of baseline differences between groups in some of the measured variables, analysis of covariance (ANCOVA) for repeated

measures was used to analyze the temporal pattern of all cardiodynamic variables, ABGs and neuroendocrine hormones. Initial baseline criterion measures were used as the covariates. When a statistically significant time effect and/or group by time interaction effect was observed, within group comparisons between discrete time points and/or between groups comparisons at each time point were done using ANCOVA with contrast analysis for obtaining appropriate post hoc custom hypotheses tests.

All statistical analyses were completed using the SAS statistical program. An alpha level of $p \leq 0.05$ was required for statistical significance.

CHAPTER 4 RESULTS

Descriptive Characteristics

The physical characteristics of HTR and control groups are presented in table 1. The two groups did not differ ($p \geq 0.05$) with respect to age (50.4 vs 50.1 yrs), height (178 vs 176 cm) and weight (85.5 vs 85 kg). Though the groups did not differ significantly in body composition, HTR had more body fat (27.5 vs 24.7 %) and less lean body mass (LBM) than the control subjects (61.5 vs 63.7 kg).

Leg Strength

One repetition maximum (1-RM) leg strength values are also shown in table 1. 1-RM bilateral knee-extension strength was significantly ($p \leq 0.05$) greater in the control group (95.0 vs 66.1 kgs). When 1-RM was calculated in relative terms ie., weight lifted per unit of LBM, the control group was also significantly ($p \leq 0.05$) stronger than HTR (3.28 vs 2.39 kg lifted/kg LBM).

Responses During the Symptom Limited-Graded Exercise Test

Hemodynamic Changes

Baseline and peak exercise values for heart rate and blood pressure during the SL-GXT are listed in table 2. Resting heart rate in HTR was 38% higher ($p \leq 0.05$) than in the control group (94 vs 68 beats/min). Peak

exercise heart rate in HTR was 57% higher than at baseline compared with a significantly higher ($p \leq 0.05$) 149% increase from baseline in the control group. Mean chronotropic reserve for HTR was 53 beats/min compared to 100 beats/min for the control group ($p \leq 0.05$).

Resting systolic blood pressure did not differ ($p \geq 0.05$) between the two groups (126 vs 126 mmHg). Peak systolic blood pressure was 15% higher ($p \leq 0.05$) in the control group than in HTR (218 vs 190 mmHg).

Diastolic blood pressure at rest (90 vs 77 mmHg) and at peak exercise (92 vs 76 mmHg) was significantly higher ($p \leq 0.05$) in HTR. The change in mean diastolic blood pressure from rest to peak exercise was -0.2 and 2.0 mmHg for the control and HTR, respectively, but the change during exercise was not significant between groups ($p \geq 0.05$).

The rate-pressure product ($\text{peak HR} \times \text{peak SBP} \times 10^{-2}$) at peak exercise was 31% greater ($p \leq 0.05$) in the control group compared to HTR (369 vs 280).

Cardiorespiratory Responses

Peak cardiorespiratory responses during the SL-GXT are further summarized in table 3. The peak $\dot{V}O_2$ achieved by HTR was 57% of the peak $\dot{V}O_2$ attained by the control group (18.3 vs 32.0 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ($p \leq 0.05$). The dissimilarity in peak $\dot{V}O_2$ was further reflected in the peak power output (Watts) at termination of the SL-GXT with HTR achieving a peak power output value that was 55% of that attained by the control group (109 vs 198 watts) ($p \leq 0.05$).

Peak respiratory exchange ratio (RER) averaged 1.16 ± 0.06 in all subjects indicating a high degree of hyperventilation during the SL-GXT. Peak RER was, however, significantly higher ($p \leq 0.05$) in the control group

(1.17 vs 1.15 for the control and HTR, respectively). Peak expired carbon dioxide ($\dot{V}CO_2$) (3.10 vs 1.73 l·min⁻¹) and peak pulmonary ventilation ($\dot{V}_E$) (97.7 vs 69.5 l·min⁻¹) were also significantly higher ($p \leq 0.05$) in the control group with HTR achieving mean $\dot{V}_E$ values that were 71% of the control group value. Peak ventilatory equivalent for $\dot{V}O_2$ ($\dot{V}_E / \dot{V}O_2$) was significantly greater ($p \leq 0.05$) in HTR (45.7 vs 37.5 l·min⁻¹) indicating that HTR ventilated more for a given level of oxygen uptake than did the controls.

Neuroendocrine Responses

Measurements of serum aldosterone (ALDO) and plasma renin activity (PRA), vasopressin (AVP), atrial natriuretic peptide (ANP), norepinephrine (NE) and angiotensin II (ANG II) were made at rest and immediately after the SL-GXT. Baseline and peak concentrations of the neuroendocrine hormones during the SL-GXT are presented in table 4.

The changes in ALDO and ANG II concentrations from rest to peak exercise are shown in figure 1. Baseline serum ALDO was not significantly different ($p \geq 0.05$) between the two groups (164.9 vs 119.9 pg/ml for HTR and control, respectively). Serum ALDO values at peak exercise (300.1 and 241.4 pg/ml for HTR and control groups respectively) were significantly greater ($p \leq 0.05$) than baseline in both groups but the group by time interaction was not significant ($p \geq 0.05$).

Baseline plasma ANG II was not significantly different ($p \geq 0.05$) between the two groups (4.1 vs 2.7 pg/ml) but HTR exhibited a trend toward higher ANG II concentrations at rest ($p = 0.07$). HTR significantly ($p \leq 0.05$) increased plasma ANG II at peak exercise but the increase was not

greater ($p \geq 0.05$) than in the control group. Peak exercise ANG II values reached 12.1 and 12.5 pg/ml for HTR and control groups, respectively.

Baseline concentrations of plasma AVP were elevated in HTR (4.2 vs 2.8 pg/ml) compared to the control group but the difference did not reach statistical significance ($p = 0.06$). Plasma AVP increased significantly ($p \leq 0.05$) in both groups during the SL-GXT but the increase was significantly greater ($p \leq 0.05$) for HTR (27.3 and 16.7 pg/ml at peak exercise for HTR and control groups, respectively).

Baseline PRA (8.3 vs 1.5 ng/ml/hr) and plasma ANP concentrations (42.9 vs 18.1 pg/ml) were significantly higher ($p \leq 0.05$) in HTR compared to the control group. Both groups significantly increased ($p \leq 0.05$) PRA and ANP concentrations at peak exercise but the magnitude of increase in PRA (200 vs 87 %) and ANP (221 vs 163 %) was greater ($p \leq 0.05$) for HTR.

Plasma NE at rest was not significantly different between the two groups (0.478 vs 0.394 ng/ml) but HTR demonstrated a trend toward higher NE at rest ($p = 0.08$). NE at peak exercise was significantly higher ($p \leq 0.05$) than baseline for both groups but the increase in NE was greater ($p \leq 0.05$) in HTR (7 vs 5 fold increase).

Pulmonary Function Tests

Pre- versus PostTransplant Pulmonary Function

The results of the resting pulmonary function tests performed before and after Tx are presented in table 5. One patient did not complete a preTx pulmonary function test, therefore, the comparisons of pre- and postTx pulmonary function tests included 10 HTR. General improvement in pulmonary function was found in all 10 HTR. Significant ($p \leq 0.05$)

improvements in pulmonary mechanics were observed with mean increases of 14.4% in forced vital capacity (FVC) and 9.7% in forced expired volume in one second (FEV₁). Total lung capacity (TLC) did not change ($p \geq 0.05$) in relation to TLC values before Tx (90.3 vs 89.2 % of predicted before and after Tx, respectively).

Pulmonary diffusion capacity (DLCO) also improved significantly following Tx ($p \leq 0.05$) with mean increases of 6.1% in whole lung DLCO and 7.8% in DLCO per unit of total lung volume (DLCO/TLC). Resting PaO₂ (92.4 before vs 101.2 mmHg after) and PaCO₂ (35.2 before vs 37.1 mmHg after) showed a trend toward improving after Tx but the changes were not significant ($p \geq 0.05$). Given the increases observed in PaO₂ and PaCO₂, however, the alveolar to arterial gradient for oxygen partial pressure improved from 12.6 before to 3.3 mmHg after Tx.

The mean whole blood cyclosporine concentration in HTR was 570 ng/ml during the month in which the postTx pulmonary function test was administered. The correlation coefficient for the relationship between the change in diffusing capacity from pre- to postTx and blood levels of cyclosporine was not significant ($p = 0.822$) ($r = 0.18$) indicating no relationship between cyclosporine immunosuppression therapy and DLCO. Furthermore, the change in DLCO from pre- to postTx was not significantly correlated with either patient age ($p = 0.335$) ($r = -0.34$) or the number of months postTx ($p = 0.792$) ($r = -0.10$). Additional correlational analysis did, however, demonstrate a high inverse relationship between smoking history (pack years) and preTx DLCO ($p = 0.009$) ($r = -0.77$) as well as postTx DLCO ($p = 0.006$) ($r = -0.80$).

Although general pulmonary function was improved in HTR following Tx, pulmonary function measures for HTR were significantly ($p \leq 0.05$) lower when compared to the control group. Absolute and relative (% of predicted) measures of diffusion capacity (DLCO, DLCO/TLC) and pulmonary mechanics (FVC, FEV₁) were higher ($p \leq 0.05$) in the control group compared to HTR, but total lung capacity (TLC) was not different ($p \geq 0.05$) between groups. Pulmonary function test values for HTR following surgery and the control group are presented in table 6.

Responses During Submaximal Exercise

Relative Exercise Intensity

The means $\pm$ SEM of physiological parameters that were considered valid indicators of differences in relative exercise intensity between the two groups are shown in table 7. There were nonsignificant ($p \geq 0.05$) differences between HTR and control groups for heart rate (% of heart rate reserve), rating of perceived exertion (RPE) and losses of plasma volume (% Δ from rest) at the conclusion of each of the exercise conditions. These data indicate that the relative exercise stimulus during the submaximal exercise tests was comparable between groups.

Arterial Blood Gas: Transplant vs Control Groups

ABG data for one HTR and one control subject had to be rejected due to technical difficulty with the ABG analysis machine. Therefore, group ABG comparisons included 10 HTR and 10 control subjects. Absolute values for PaO₂, PaCO₂ and pH as a function of experimental treatments are presented in tables 8 and 9.

The temporal patterns of arterial PO_2 , PCO_2 and pH during 10 minutes of constant load cycle exercise at 40% of peak power output are also illustrated in figure 2. Baseline PaO_2 was not significantly different ($p \geq 0.05$) between HTR and control groups. During 10 minutes of constant load cycle exercise at 40% of peak power output, arterial PO_2 decreased significantly ($p \leq 0.05$) below resting values within 1 minute after commencement of exercise in both groups. PaO_2 returned to values that were not significantly different from rest at 2.5 minutes in the control group but PaO_2 continued to decrease linearly over time in HTR. The group by time interaction, however, was not significant ($p \geq 0.05$). Baseline arterial PCO_2 levels were significantly ($p \leq 0.05$) lower in HTR and continued to decrease during exercise with the temporal pattern of PaCO_2 becoming significantly different ($p \leq 0.05$) from baseline by 4 minutes after the onset of exercise while the control group remained isocapnic. Nonetheless, the group by time interaction for PaCO_2 was not significant ($p \geq 0.05$). Arterial pH was similar ($p \geq 0.05$) at rest in both groups. The pH response during exercise was also similar ($p \geq 0.05$) with values decreasing significantly ($p \leq 0.05$) below resting values by the third minute of exercise in both groups.

The temporal patterns of PaO_2 , PaCO_2 and pH during 10 minutes of constant load cycle exercise at 70% of peak power output are shown in figure 3. Arterial PO_2 decreased significantly ($p \leq 0.05$) below resting values within 1 minute after commencement of work in both groups. After reaching the nadir at 1.5 minutes, PaO_2 returned to values not significantly different ($p \geq 0.05$) from baseline by 2.5 minutes in the control group but PaO_2 continued to decrease linearly over time in HTR. The

group by time interaction was significant ($p \leq 0.05$) with respect to the temporal pattern of PaO_2 , with PaO_2 values in HTR becoming significantly different ($p \leq 0.05$) from the control group after the third minute of exercise. The group by time interaction was also significant ($p \leq 0.05$) with respect to the temporal pattern of PaCO_2 . The magnitude of decrease in PaCO_2 values in HTR became significantly different ($p \leq 0.05$) from the control group by 2 minutes of exercise and continued to decline throughout the exercise bout. Arterial pH decreased linearly over time in both groups and was significantly different ($p \leq 0.05$) from resting values after 2 minutes of exercise. The group by time interaction for arterial pH during cycle exercise at 70% of peak power was not significant ($p \geq 0.05$).

Arterial Blood Gas Profile of the Transplant Group

Considerable variability was present in mean arterial PO_2 , PCO_2 and pH values during both submaximal exercise conditions for HTR. To further examine possible sources of variability, HTR were separated into groups; those with normal (NL-DLCO; $n=5$) and those with below normal pulmonary diffusion capacity (LO-DLCO; $n=5$). American Thoracic Society guidelines for pulmonary function testing were used as the criteria for group assignments (NL-DLCO $\geq 70\%$ of predicted reference values) (Miller et al., 1983). Individual HTR data for arterial PO_2 , PCO_2 and pH during exercise at 70% of peak power output are plotted in figures 4, 5 and 6, respectively. These HTR data were then subjected to further analysis. Baseline arterial PO_2 , PCO_2 and pH were not significantly different ($p \geq 0.05$) between the two groups. During cycle exercise at 40% of peak power, group by time interactions for arterial PO_2 , PCO_2 and pH were not significant ($p \geq 0.05$). During exercise at 70% of peak power, however, the

group by time interactions for arterial PO_2 , PCO_2 and pH were each significant. Arterial PO_2 in the LO-DLCO group became significantly ($p \leq 0.05$) lower than the NL-DLCO group by 1.5 minutes after the onset of exercise and continued to decrease over time, with PaO_2 dropping below 70 mmHg in two HTR. Arterial PCO_2 in the LO-DLCO group decreased significantly ($p \leq 0.05$) more than the NL-DLCO group by 3.5 minutes of exercise and arterial pH dropped more rapidly in the LO-DLCO group, becoming significantly different from the NL-DLCO group by 4.5 minutes after the commencement of exercise. Additional correlational analysis revealed highly significant inverse relationships between resting DLCO and the transient hypoxia experienced during cycle exercise at 40% ($p=0.014$)($r=-0.94$) and 70% ($p=0.001$)($r=-0.97$) of peak power output in HTR.

Exercise Hyperpnea After Cardiac Transplantation

Additional analyses of ABG alterations during the transition from rest to steady state exercise were performed on the NL-DLCO group and a matched control group ($n=5$) to determine the effect of Tx on pulmonary ventilation in HTR with DLCO. Figure 7 shows the ABG data for the NL-DLCO group and control group during cycle exercise at 40% of peak power. The resting values for PaO_2 , PaCO_2 and pH were not significantly different ($p \geq 0.05$) between groups. The absolute ABG changes during exercise did not vary significantly ($p \geq 0.05$) between the control and transplant groups. Furthermore, PaCO_2 did not significantly vary ($p \geq 0.05$) from baseline values during exercise. These data indicate that cardiac afferent reflexes are not essential for adequate pulmonary ventilation at the onset of exercise and that the exercise hyperpnea was regulated at an isocapnic level.

Cardiac Output

Cardiac output data for one transplant patient had to be rejected because the Impedance Cardiograph signal was not of sufficient quality to enable any analysis to be performed. Therefore, data from the matched control subject were also excluded from the analysis. Because the control and HTR were closely matched with respect to age, height and weight, cardiac output and stroke volume values are reported without correction for body surface area, ie. cardiac and stroke volume index. Absolute values for cardiac output, stroke volume and heart rate during the two submaximal testing conditions are shown in tables 10 and 11. The relative changes (% Δ from baseline) in cardiac output, stroke volume and heart rate during exercise at 40% of peak power output are illustrated in figure 8. Baseline cardiac output was within a normal range for HTR but significantly less ($p \leq 0.05$) than the control group (4.94 and 4.58 for the control and HTR, respectively). Both groups increased cardiac output significantly ($p \leq 0.05$) above resting values within 30 seconds after commencement of exercise and reached values by the 10th minute of exercise that were 109 and 94% greater than baseline for the control and HTR, respectively. Consistent with results from the SL-GXT, baseline heart rate was significantly higher ($p \leq 0.05$) in the HTR (105 vs 74 bpm). Both groups increased heart rate significantly ($p \leq 0.05$) above baseline at all measurement periods but the magnitude of increase was greater ($p \leq 0.05$) in the control group. The peak increase in heart rate during cycle exercise at 40% of peak power was 17 and 54% greater than baseline for HTR and control groups, respectively. Baseline stroke volume was significantly lower ($p \leq 0.05$) at rest in HTR (45 vs 68 ml). Stroke volume increased

significantly ($p \leq 0.05$) above baseline after 30 seconds of exercise in HTR but stroke volume in the control group did not become significantly greater ($p \leq 0.05$) than baseline until 2 minutes after the commencement of exercise. Stroke volume during exercise at 40% of peak power output increased 38 and 61% above baseline in the control and HTR, respectively.

Relative increases ($\% \Delta$ from baseline) in cardiac output, stroke volume and heart rate during 10 minutes of constant load cycle exercise at 70% of peak power output are shown in figure 9. Cardiac output was significantly increased ($p \leq 0.05$) above baseline in both groups at each measurement period. The increase in cardiac output was significantly greater ($p \leq 0.05$) in the control group compared to HTR by 5 minutes after the onset of exercise. Peak cardiac output at 10 minutes of exercise was 158 and 132% greater than baseline for control and HTR, respectively. Stroke volume increased rapidly at the onset of exercise in HTR and was significantly greater ($p \leq 0.05$) than baseline at each measurement period during exercise. Stroke volume did not become significantly greater ($p \leq 0.05$) than baseline in the control group until 2.5 minutes after the onset of exercise. The relative increase in stroke volume was significantly greater ($p \leq 0.05$) in HTR at all measurement periods compared to the control group. Peak stroke volume was 33 and 69% greater than baseline in the control and HTR, respectively. Exercise heart rate for both groups was significantly greater ($p \leq 0.05$) than baseline at all measurement periods. During the first minute of exercise at 70% of peak power, heart rate in HTR increased only 7% compared with a 60% increase in the control group ($p \leq 0.05$). Heart rate in HTR continued to increase slowly during the remainder of the exercise test and at 10 minutes, heart rate was only 33%

higher than at baseline. Heart rate in the control group increased 98% by 10 minutes after the onset of exercise.

Neuroendocrine Hormones

Absolute values for plasma AVP, NE, ANP and PRA during the two submaximal exercise conditions are presented in tables 12 and 13. Data from all subjects (n=11 HTR; n=11 control) were included in the neuroendocrine hormone analysis with the exception of NE. Blood samples were assayed for plasma NE in 6 control subjects and all 11 HTR.

Vasopressin

The changes in AVP during cycle exercise at 40 and 70% of peak power output and the SL-GXT are illustrated in figures 10 and 11. Baseline AVP was not significantly different ($p \geq 0.05$) between the two groups (3.11 vs 3.55 pg/ml for control and HTR respectively). During 10 minutes of constant load cycle exercise at 40% of peak power, AVP increased to levels significantly greater ($p \leq 0.05$) than baseline by 4 minutes after the commencement of exercise in both groups but the group by time interaction was not significant ($p \geq 0.05$).

The resting concentration of AVP prior to the second submaximal bout did not vary significantly ($p \geq 0.05$) from initial baseline values. During cycle exercise at 70% of peak power output, AVP increased significantly ($p \leq 0.05$) above baseline values within 2 minutes after the onset of work in both groups. AVP continued to increase linearly over time in both groups but the increase in AVP in HTR was greater ($p \leq 0.05$) than the control group by the sixth minute after the onset of exercise.

Plasma Renin Activity

Changes in PRA during submaximal exercise and the SL-GXT are illustrated in figures 12 and 13. Baseline PRA values were significantly greater ($p \leq 0.05$) in HTR (8.98 and 1.10 ng/ml/hr for HTR and control groups, respectively). During cycle exercise at 40% of peak power, PRA became significantly greater than baseline 6 minutes after the onset of exercise and continued to increase over time but the changes were similar ($p \geq 0.05$) for both groups.

Resting PRA did not vary significantly ($p \geq 0.05$) between the two exercise tests for either group. During cycle exercise at 70% of peak power output, PRA values were significantly greater ($p \leq 0.05$) than rest at all measurement periods in both groups. The group by time interaction was significant and post hoc contrast analysis showed that the magnitude of increase by 4 minutes after the onset of exercise was significantly greater ($p \leq 0.05$) in HTR.

Atrial Natriuretic Hormone

Changes in ANP during submaximal exercise and the SL-GXT are depicted in figures 14 and 15. ANP at rest was significantly elevated ($p \leq 0.05$) in HTR (41.45 and 20.19 pg/ml for HTR and control groups, respectively). ANP increased at the onset of cycle exercise at 40% of peak power and was significantly ($p < 0.05$) greater than baseline by 8 minutes after the onset of exercise in both groups and the group by time interaction was not significant ($p \geq 0.05$).

Resting ANP prior to the second exercise bout was not significantly different ($p \geq 0.05$) from initial basal levels. During cycle exercise at 70% of peak power output, ANP values were significantly greater ($p \leq 0.05$) than

baseline at all measurement periods in both groups but the magnitude of increase was significantly greater ($p \leq 0.05$) in HTR compared to the control group by 8 minutes after the onset of exercise.

Norepinephrine

Changes in NE during exercise at 40 and 70% of peak power output are shown in figures 16 and 17. Basal NE values did not differ significantly ($p \geq 0.05$) between groups (0.396 vs 0.468 ng/ml for the control and HTR, respectively). Plasma NE concentrations were significantly ($p \leq 0.05$) elevated above resting levels in both groups by four minutes after the onset of cycle exercise at 40% of peak power output. The group by time interaction, however, was not significant ($p > 0.05$).

The resting concentrations of NE prior to the second bout of submaximal cycle exercise did not vary significantly ($p \geq 0.05$) from initial resting values. Both groups increased ($p \leq 0.05$) plasma NE concentrations above baseline within 2 minutes after the onset of cycle exercise at 70% of peak power output. The group by time interaction was significant ($p \leq 0.05$) and post hoc analysis revealed that the magnitude of NE elevation was greater ($p \leq 0.05$) in HTR at each measurement period (minute 2, 4, 6 and 10).

Table 1. Physical characteristics of the Control and Transplant groups.

VARIABLE	CONTROL	TRANSPLANT
MALES	10	10
FEMALES	1	1
AGE (yrs)	50.4±13.9	50.1±13.7
HEIGHT (cm)	178.2±6.5	176.2±9.7
WEIGHT (kg)	85.5±15.2	85.0±13.8
BODY FAT (%)	24.7±6.0	27.5±5.6
LEAN BODY MASS (kg)	63.7±8.9	61.5±9.7
1-RM LEG STRENGTH ^a (kg)	95.0±19.4	66.1±9.6*

values are mean ± standard deviation

^a knee-extension (quadriceps) strength

*p≤0.05 Transplant vs Control

Table 2. Heart rate and blood pressure values for both groups at rest and peak exercise during the symptom limited-graded exercise test.

VARIABLE	CONTROL	TRANSPLANT
REST HEART RATE ^a (beats/min)	68±7	94±10*
PEAK HEART RATE (beats/min)	168±15	147±12*
Δ BETWEEN REST AND PEAK	100±13	53±12\$
% Δ	149±25	57±17\$
REST SYSTOLIC PRESSURE ^a (mmHg)	126±12	126±9
PEAK SYSTOLIC PRESSURE (mmHg)	218±24	190±19*
Δ BETWEEN REST AND PEAK	93±24	64±21\$
% Δ	76±24	51±19\$
REST DIASTOLIC PRESSURE ^a (mmHg)	77±5	90±6*
PEAK DIASTOLIC PRESSURE (mmHg)	76±7	92±12*
Δ BETWEEN REST AND PEAK	-0.2±8	2.0±8
% Δ	0.1±12	2.2±9
PEAK RATE-PRESSURE PRODUCT ^b	369±43	280±43*

values are mean ± standard deviation

^a measurement taken prior to SL-GXT with subject seated

^b peak heart rate x peak systolic pressure x 10⁻²

* p≤0.05 transplant vs control

\$ p≤0.05 the change from rest to peak different from control

Table 3. Peak cardiorespiratory responses of the Control and Transplant groups during the symptom limited-graded exercise test.

VARIABLE	CONTROL	TRANSPLANT
$\dot{V}O_2^a$ (ml·kg ⁻¹ ·min ⁻¹)	32.0±8.7	18.3±3.3*
$\dot{V}O_2$ (L·min ⁻¹)	2.69±0.74	1.52±0.24*
$\dot{V}CO_2^b$ (L·min ⁻¹)	3.10±0.81	1.73±0.28*
$\dot{V}_E^c$ (L·min ⁻¹)	97.67±19.80	69.50±16.80*
$\dot{V}_E/\dot{V}O_2$	37.55±8.97	45.74±9.43*
RESPIRATORY EXCHANGE RATIO	1.17±0.06	1.15±0.05*
PEAK POWER (Watts)	198±47	109±20*
RATING OF PERCEIVED EXERTION ^d	19.5±0.8	19.4±0.8

values are mean ± standard deviation

^a peak systemic oxygen consumption

^b peak expired carbon dioxide

^c peak expired pulmonary ventilation (STPD)

^d Borg (1962) Scale

* p≤0.05 transplant vs control

Table 4. Aldosterone (ALDO), angiotensin II (ANGII), vasopressin (AVP), plasma renin activity (PRA), atrial natriuretic peptide (ANP) and norepinephrine (NE) at rest and immediately after the SL-GXT.

VARIABLE	GROUP	REST	PEAK-EXERCISE
ALDO (pg/ml)	CONTROL TRANSPLANT	119.8±68.7 164.9±65.8	241.4±135.9* 300.1±148.2*
ANGII (pg/ml)	CONTROL TRANSPLANT	2.7±1.5 4.1±2.0	12.5±7.1* 12.1±6.1*
AVP (pg/ml)	CONTROL TRANSPLANT	2.8±0.9 4.2±1.9	16.7±3.2* 27.3±7.3*\$
PRA (ng/ml/hr)	CONTROL TRANSPLANT	1.5±0.8 8.3±6.0 ⁺	2.8±2.1* 24.8±12.0*\$
ANP (pg/ml)	CONTROL TRANSPLANT	18.1±4.2 42.9±9.5 ⁺	47.6±17.0* 137.5±60.0*\$
NE (pg/ml)	CONTROL TRANSPLANT	394±29 478±26	2,706±639* 3,869±470*\$

values are mean ± standard deviation

⁺ p≤0.05 transplant vs control at baseline

* p≤0.05 vs rest

\$ p≤0.05 the change from rest different from control

Table 5. Pulmonary function test measurements and resting blood gas values^a of 11 patients before and after orthotopic heart transplantation.

PATIENT NO.	DLCOsb (%)		DLCO/TLCsb (%)		PaO ₂ (mmHg)		PaCO ₂ (mmHg)		CYCLOSPORINE ^b (ng/ml)
	pre	post	pre	post	pre	post	pre	post	
1	92	96	108	120	98	--	35	--	590
2	40	51	51	65	109	105	23	36	584
3	99	95	104	96	92	102	38	38	417
4	48	59	54	62	96	98	34	36	514
5	84	83	81	103	99	101	34	38	729
6	78	89	84	91	88	104	43	37	500
7	95	89	114	98	102	101	34	36	568
8	67	84	75	90	81	99	37	37	602
9	35	52	31	49	67	93	44	35	601
10	67	68	73	78	92	103	34	40	600
11	--	68	--	80	--	104	--	38	542
MEAN	70.3	76.3	77.5	84.7	92.4	101.2	35.2	37.1	570
SD	23.5	18.0	26.8	20.5	13.6	4.1	5.6	1.8	77
p value	≤0.05		≤0.05		NS		NS		

^a values are expressed as percentage of the predicted value

^b whole blood levels determined within one month of the post-transplant pulmonary function test

DLCOsb (single breath lung diffusion capacity)

DLCO/TLCsb (diffusion capacity per unit total lung volume)

Table 5 continued.

PATIENT No.	FVC (%)		FEV ₁ (%)		TLCsb (%)		SMOKING HISTORY ^b (pack-years)
	pre	post	pre	post	pre	post	
1	70	95	84	92	86	87	0
2	74	85	53	61	85	81	75
3	74	95	90	105	84	86	10
4	82	100	91	110	88	92	30
5	81	83	96	97	102	89	0
6	102	102	118	108	100	105	11
7	63	85	67	91	87	87	0
8	60	88	68	95	69	70	0
9	72	82	39	42	113	109	40
10	73	80	81	83	89	86	0
11	--	88	--	99	--	76	6
MEAN	75.1	89.4	78.7	89.4	90.3	89.2	15.6
SD	11.7	7.5	22.7	20.7	12.0	11.3	
p value	≤0.05		≤0.05		NS		

^a values are expressed as percentage of the predicted value

^b packs of cigarettes per day × years of smoking

FVC (forced vital capacity)

FEV₁ (volume expired in first second)

TLCsb (total lung capacity, single breath technique)

Table 6. Pulmonary function values^a of the control group and the transplant group after cardiac transplantation.

VARIABLE	CONTROL	TRANSPLANT
DLCO _{sb} (ml·min ⁻¹ ·mmHg)	29.20±4.39	20.74±4.21*
% predicted	106.2±10.4	76.3±18.0*
DLCO/TLC _{sb}	4.51±0.75	3.75±1.13*
% predicted	99.5±12.3	84.7±20.5*
FVC (L)	4.75±0.87	4.13±0.66*
% predicted	97.1±9.3	89.4±7.5*
FEV ₁ (L)	3.74±0.71	3.03±0.88*
% predicted	104.6±12.3	89.4±20.7*
TLC _{sb} (L)	6.50±0.64	5.74±1.08
% predicted	94.1±9.4	88.0±11.3

values are mean ± standard deviation

^a absolute values are liters BTPS

DLCO_{sb} (single breath lung diffusion capacity)

DLCO/TLC_{sb} (diffusion capacity per unit total lung volume)

FVC (forced vital capacity)

FEV₁ (volume expired in first second of FVC))

TLC_{sb} (total lung capacity single breath technique)

* p≤0.05 transplant vs control

Table 7. Heart rate^a, rating of perceived exertion and plasma volume changes^b during submaximal and maximal exercise tests.

VARIABLE	GROUP	% PEAK POWER		
		40%	70%	100%
HEART RATE (% HRR)	CONTR	46.4±8.6	81.2±6.4	
	TRANS	51.9±16.3	81.6±7.9	
PERCEIVED EXERTION ^c	CONTR	12.2±1.2	15.1±1.1	19.5±0.8
	TRANS	12.5±1.2	16.6±1.2	19.4±0.8
%Δ PLASMA VOLUME	CONTR	-4.6±3.4	-10.8±2.4	-12.9±5.6
	TRANS	-5.7±4.3	-10.1±3.8	-14.1±5.5

values are mean ± standard deviation

^a heart rate expressed as percentage of heart rate reserve

^b percentage loss in plasma volume (rest-exercise)

^c Borg (1962) Scale

Table 8. Changes in arterial PO₂, PCO₂ and pH during 10 minutes of constant load cycle exercise at 40% of peak power output.

TIME (min)	PaO ₂ (mmHg)		PaCO ₂ (mmHg)		pH	
	CTRa	TX ^b	CTR	TX	CTR	TX
REST	101.8 ±2.3	101.1 ±4.1	38.6 ±1.6	37.2 ⁺ ±1.4	7.41 ±0.01	7.42 ±0.02
.5	102.1 ±3.3	99.9 ±4.3	38.4 ±1.1	37.2 ±1.7	7.41 ±0.01	7.42 ±0.02
1	98.9* ±2.7	97.4* ±3.9	38.2 ±1.5	37.3 ±1.9	7.41 ±0.01	7.41 ±0.02
1.5	99.1* ±1.9	96.0* ±5.9	39.3 ±1.6	37.1 ±1.9	7.41 ±0.02	7.41 ±0.02
2	99.7* ±1.8	95.1* ±6.5	38.8 ±1.2	36.9 ±2.0	7.40 ±0.02	7.40 ±0.02
2.5	100.4 ±1.6	94.9* ±7.2	39.0 ±1.5	36.6 ±2.1	7.40 ±0.01	7.39 ±0.03
3	102.0 ±1.6	94.7* ±7.6	38.7 ±1.5	36.6 ±2.1	7.39* ±0.02	7.39* ±0.03
3.5	101.8 ±1.0	94.4* ±7.3	38.8 ±1.5	36.5 ±2.1	7.39* ±0.02	7.39* ±0.02
4	101.8 ±0.9	94.9* ±8.7	38.8 ±1.4	36.1* ±2.2	7.39* ±0.02	7.38* ±0.02
4.5	101.8 ±1.9	93.5* ±8.4	38.9 ±1.4	36.0* ±2.1	7.39* ±0.02	7.38* ±0.02
5	100.9 ±1.3	93.4* ±8.8	38.7 ±1.5	35.8* ±2.4	7.38* ±0.01	7.38* ±0.02
6	100.9 ±1.4	92.9* ±8.9	38.7 ±2.3	35.9* ±2.4	7.39* ±0.02	7.38* ±0.03
8	101.3 ±1.6	92.8* ±9.9	38.0 ±0.8	35.4* ±2.3	7.39* ±0.02	7.38* ±0.03
10	101.7 ±1.9	92.6* ±10.4	38.0 ±1.1	35.2* ±2.5	7.39* ±0.02	7.37* ±0.03

values are mean ± standard deviation

^a control group (n=10)

^b transplant group (n=10)

⁺ p≤0.05 transplant vs control at baseline

* p≤0.05 vs rest

Table 9. Changes in arterial PO₂, PCO₂ and pH during 10 minutes of constant load cycle exercise at 70% of peak power output.

TIME (min)	PaO ₂ (mmHg)		PaCO ₂ (mmHg)		pH	
	CTR ^a	TX ^b	CTR	TX	CTR	TX
REST	101.5 ±2.7	101.6 ±4.3	37.9 ±1.5	36.8 ⁺ ±1.1	7.41 ±0.02	7.42 ±0.03
.5	99.7 ±2.7	98.9 ±4.3	37.4 ±1.4	36.5 ±1.1	7.41 ±0.02	7.42 ±0.02
1	98.1* ±2.7	97.6* ±5.3	38.1 ±1.3	36.6 ±1.5	7.40 ±0.01	7.41 ±0.02
1.5	97.9* ±1.4	94.8* ±6.0	38.3 ±1.3	36.5 ±1.8	7.40 ±0.03	7.40 ±0.02
2	98.7* ±1.5	94.0* ±6.6	38.4 ±1.5	36.2\$ ±2.0	7.39 ±0.03	7.39 ±0.03
2.5	99.4 ±1.7	93.5* ±7.4	38.4 ±1.4	36.2\$ ±2.0	7.38* ±0.02	7.39* ±0.02
3	100.5 ±2.1	93.4* ±7.9	38.5 ±2.0	35.7*\$ ±2.2	7.38* ±0.02	7.38* ±0.02
3.5	100.6 ±2.2	91.8*\$ ±6.8	38.4 ±2.3	35.2*\$ ±2.4	7.37* ±0.02	7.37* ±0.03
4	101.0 ±2.0	92.1*\$ ±8.3	37.9 ±2.0	34.9*\$ ±2.1	7.37* ±0.02	7.37* ±0.03
4.5	101.6 ±2.5	90.8*\$ ±8.9	37.8 ±1.8	34.6*\$ ±2.2	7.37* ±0.02	7.36* ±0.03
5	100.7 ±2.6	90.2*\$ ±9.8	37.8 ±1.8	34.6*\$ ±2.4	7.36* ±0.02	7.36* ±0.03
6	100.1 ±2.1	89.3*\$ ±10.4	37.5 ±2.1	34.2*\$ ±2.4	7.37* ±0.03	7.35* ±0.03
8	100.2 ±2.6	88.7*\$ ±11.9	36.9* ±1.7	33.7*\$ ±2.6	7.36* ±0.03	7.34* ±0.03
10	100.0 ±2.5	87.5*\$ ±13.1	36.9* ±1.7	33.3*\$ ±2.5	7.36* ±0.03	7.33* ±0.03

values are mean ± standard deviation

^a control group (n=10)

^b transplant group (n=10)

⁺ p≤0.05 transplant vs control at baseline

* p≤0.05 vs rest

\$ p≤0.05 the change from rest different from control

Table 10. Changes in cardiac output (Q), stroke volume (SV) and heart rate (HR) during 10 minutes of constant load cycle exercise at 40% of peak power output.

TIME (min)	CARDIAC OUTPUT (L/min)		STROKE VOLUME (ml)		HEART RATE	
	CTRa	TX ^b	CTR	TX	CTR	TX
REST	4.94 ±0.40	4.58 ⁺ ±0.27	68 ±9	45 ⁺ ±4	74 ±11	105 ⁺ ±8
.5	5.94* ±1.00	5.34* ±0.56	63 ±8	49* ±7	97* ±12	108*\$ ±7
1	6.96* ±1.00	6.00* ±0.71	68 ±6	55* ±9	103* ±12	110*\$ ±8
1.5	7.47* ±1.17	6.38* ±0.76	71 ±7	57* ±10	106* ±12	111*\$ ±9
2	7.93* ±1.21	6.80* ±1.04	73* ±8	60* ±13	108* ±13	113*\$ ±8
2.5	8.22* ±1.30	7.14* ±1.01	76* ±7	63* ±13	108* ±13	113*\$ ±9
3	8.62* ±1.36	7.58* ±1.01	80* ±8	66* ±12	110* ±14	115*\$ ±9
3.5	8.89* ±1.42	7.75* ±1.05	81* ±9	67* ±12	110* ±14	117*\$ ±9
4	9.16* ±1.45	7.72* ±1.2	84* ±10	66* ±13	110* ±14	118*\$ ±10
4.5	9.18* ±1.32	7.91* ±1.19	84* ±8	67* ±13	111* ±14	119*\$ ±9
5	9.56* ±1.54	8.31* ±1.28	86* ±10	69* ±14	110* ±13	120*\$ ±9
6	10.01* ±1.37	8.50* ±1.30	90* ±10	70* ±14	111* ±13	121*\$ ±10
8	10.15* ±1.28	8.75* ±1.24	91* ±9	72* ±14	112* ±13	122*\$ ±11
10	10.34* ±1.27	8.75* ±1.27	92* ±9	72* ±14	112* ±12	123*\$ ±11

values are mean ± standard deviation

a control group (n=10)

b transplant group (n=10)

+ p<0.05 transplant vs control at baseline

* p≤0.05 vs rest

\$ p≤0.05 the change from rest different from control

Table 11. Changes in cardiac output (Q), stroke volume (SV) and heart rate (HR) during 10 minutes of constant load cycle exercise at 70% of peak power output.

TIME (min)	CARDIAC OUTPUT (L/min)		STROKE VOLUME (ml)		HEART RATE	
	CTR ^a	TX ^b	CTR	TX	CTR	TX
REST	5.16 ±0.44	4.59 ⁺ ±0.24	68 ±9	45 ⁺ ±4	76 ±10	106 ⁺ ±11
.5	6.90* ±1.12	5.87* ±0.59	67 ±9	53* ±8	108* ±13	111*\$ ±11
1	7.76* ±1.20	6.76* ±0.70	69 ±8	60* ±10	121* ±15	114*\$ ±11
1.5	8.54* ±1.33	7.42* ±0.99	70 ±7	64* ±12	130* ±16	117*\$ ±11
2	9.40* ±1.45	7.90* ±1.20	72 ±7	65* ±15	132* ±15	121*\$ ±14
2.5	10.16* ±1.55	8.46* ±1.11	75* ±9	69* ±13	136* ±15	125*\$ ±13
3	10.57* ±1.57	8.64* ±1.37	77* ±8	68* ±15	137* ±14	128*\$ ±13
3.5	11.04* ±1.61	8.91* ±1.32	79* ±9	69* ±14	139* ±14	130*\$ ±13
4	11.51* ±1.53	9.29* ±1.47	82* ±9	70* ±15	141* ±15	132*\$ ±14
4.5	11.70* ±1.41	9.54* ±1.38	83* ±8	72* ±15	143* ±16	133*\$ ±12
5	12.33* ±1.51	9.75*\$ ±1.36	87* ±10	72* ±14	144* ±15	135*\$ ±12
6	12.83* ±1.48	10.02*\$ ±1.40	88* ±11	74* ±15	146* ±15	137*\$ ±12
8	12.99* ±1.40	10.32*\$ ±1.41	90* ±11	75* ±14	148* ±15	138*\$ ±12
10	13.31* ±1.64	10.53*\$ ±1.60	90* ±13	75* ±15	150* ±15	140*\$ ±12

values are mean ± standard deviation

^a control group (n=10)

^b transplant group (n=10)

⁺ p≤0.05 transplant vs control at baseline

* p≤0.05 vs rest

\$ p≤0.05 the change from rest different from control

Table 12. Changes in plasma vasopressin (AVP), plasma renin activity (PRA), atrial natriuretic peptide (ANP) and norepinephrine (NE) during 10 minutes of constant load cycle exercise at 40% of peak power output.

VARIABLE	GROUP	TIME (min)					
		REST	2	4	6	8	10
AVP (pg/ml)	CONTROL	3.11±0.85	3.84±0.97	4.39±1.00*	4.98±1.23*	5.26±1.28*	5.45±1.31*
	TRANSPLANT	3.55±2.28	5.33±4.15	5.83±4.14*	6.66±4.35*	7.53±5.18*	8.12±5.73*
PRA (ng/ml/hr)	CONTROL	1.10±0.70	1.31±0.98	1.41±1.02	1.48±1.04*	1.66±1.15*	1.81±1.26*
	TRANSPLANT	8.98±8.82 ⁺	9.54±8.54	9.84±8.47	10.79±9.34*	11.060±9.26*	12.62±10.64*
ANP (pg/ml)	CONTROL	20.19±6.00		25.15±6.90		30.34±8.40*	33.08±8.60*
	TRANSPLANT	41.45±13.00 ⁺		46.29±12.00		55.56±18.00*	68.70±27.00*
NE (pg/ml)	CONTROL	396±32		773±75*	871±65*		1,044±99*
	TRANSPLANT	468±28		1,012±98*	1,121±99*		1,336±113*

values are mean ± standard deviation

+ p≤0.05 transplant vs control at baseline

* p≤0.05 vs rest

\$ p≤0.05 the change from rest different from control

Table 13. Changes in plasma vasopressin (AVP), plasma renin activity (PRA), atrial natriuretic peptide (ANP) and norepinephrine (NE) during 10 minutes of constant load cycle exercise at 70% of peak power output.

VARIABLE	GROUP	TIME (min)				
		REST	2	4	6	10
AVP (pg/ml)	CONTROL	3.35±1.43	5.95±2.74*	8.04±3.31*	9.89±3.98*	10.65±3.22*
	TRANSPLANT	3.55±2.14	8.46±6.20*	11.57±5.74*	15.80±6.48*\$	17.86±6.07*\$
PRA (ng/ml/hr)	CONTROL	1.14±0.69	1.38±0.95	1.56±0.98*	1.72±1.03*	1.98±1.25*
	TRANSPLANT	9.12±9.57+	12.82±10.42*	15.10±13.50*\$	16.31±13.8*\$	18.76±16.20*\$
ANP (pg/ml)	CONTROL	21.62±6.40		30.30±5.90*		35.78±9.70*
	TRANSPLANT	45.02±14.99+		58.25±12.90*		80.39±20.00*\$
NE (pg/ml)	CONTROL	393±27	912±81*	1,079±131*	1,368±170*	1,607±257*
	TRANSPLANT	488±24	1,445±148*\$	1,779±171*\$	2,241±180*\$	2,611±216*\$

values are mean ± standard deviation

+ p≤0.05 transplant vs control at baseline

* p≤0.05 vs rest

\$ p≤0.05 the change from rest different from control

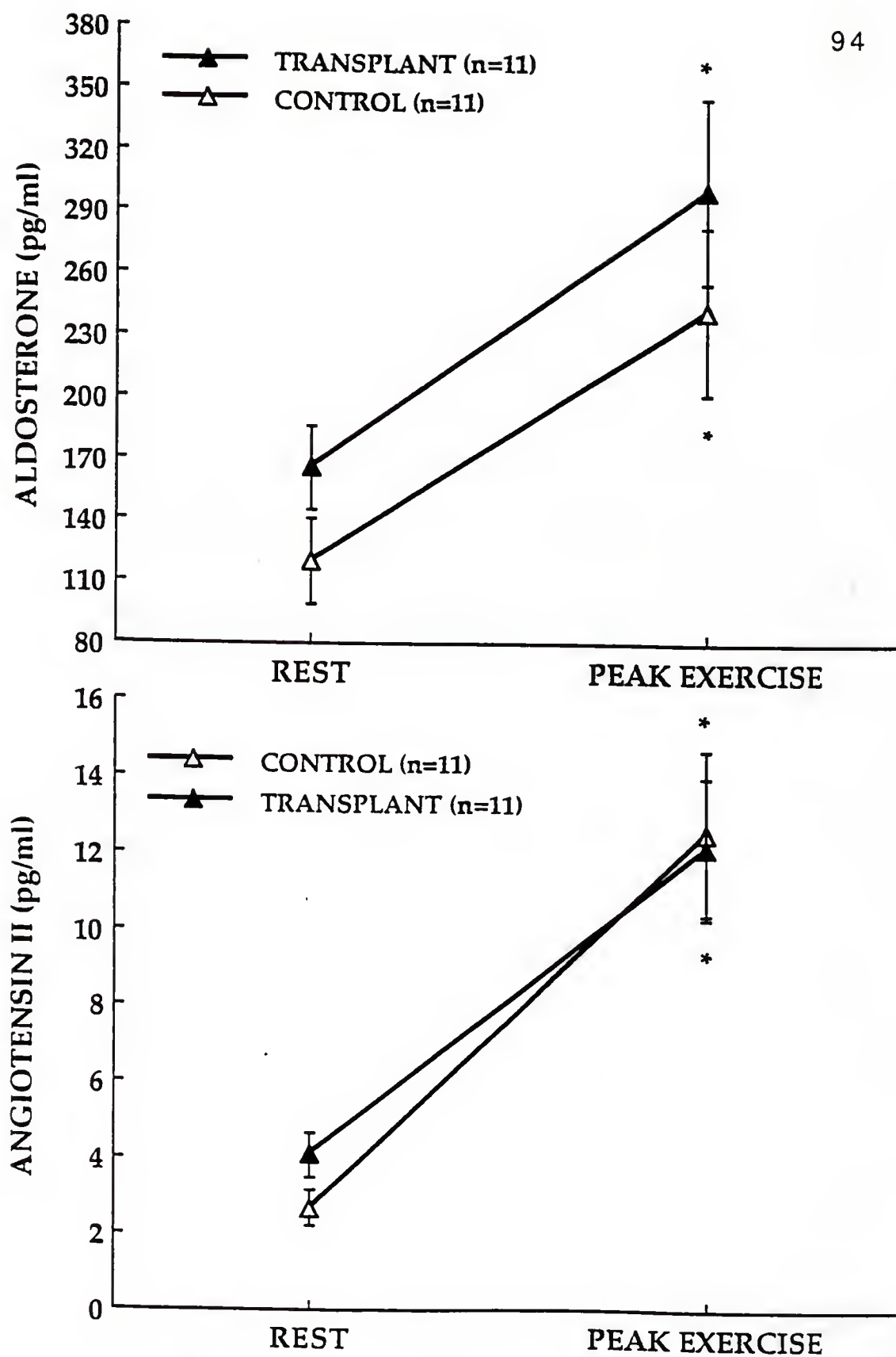


Figure 1. Serum aldosterone (top) and plasma angiotensin II at rest and immediately after the SL-GXT. (mean±SEM) * $p \leq 0.05$ vs rest

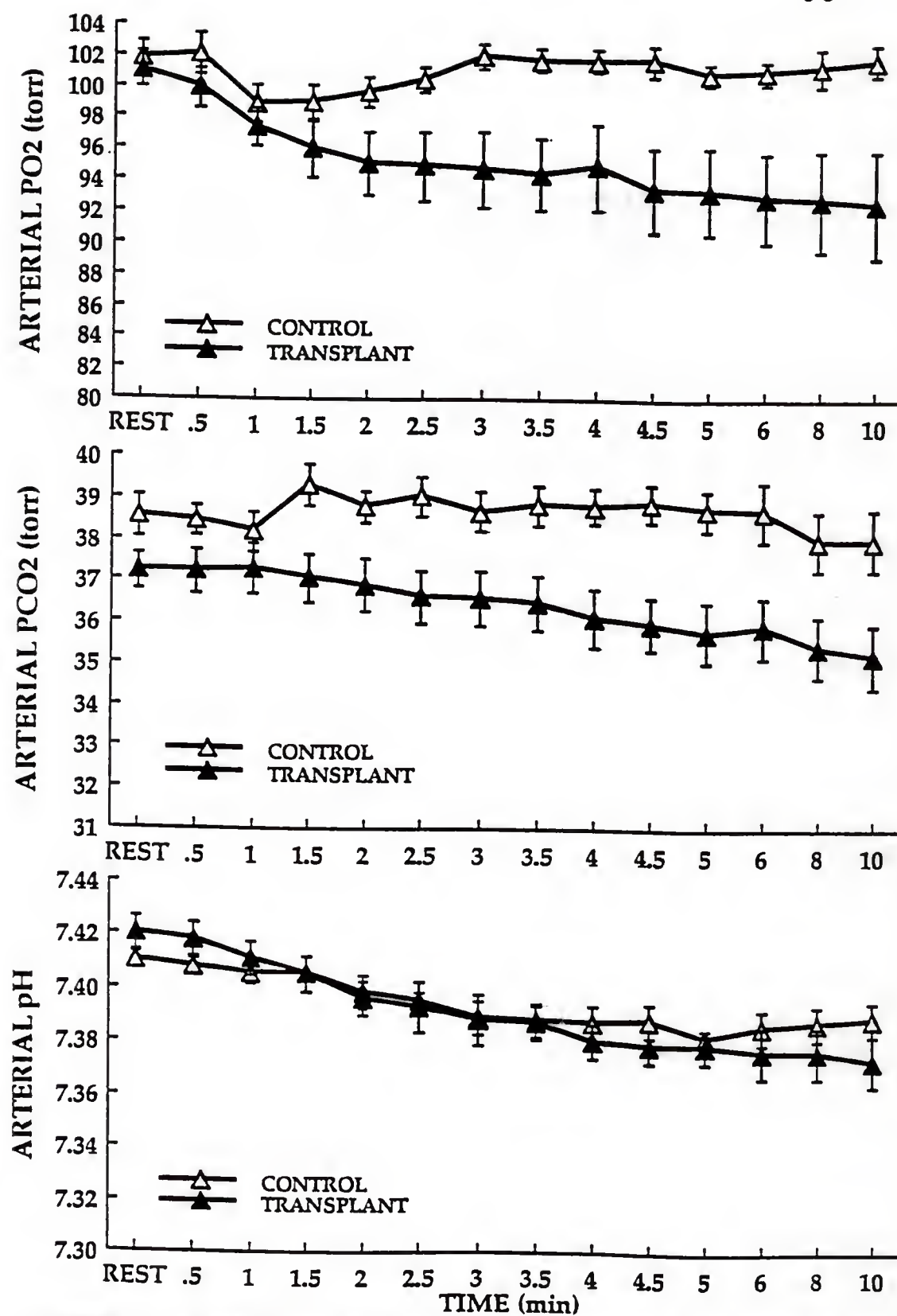


Figure 2. Changes in arterial PO₂, PCO₂ and pH during constant load cycle exercise at 40% of peak power. (n=10 for transplant and control groups)

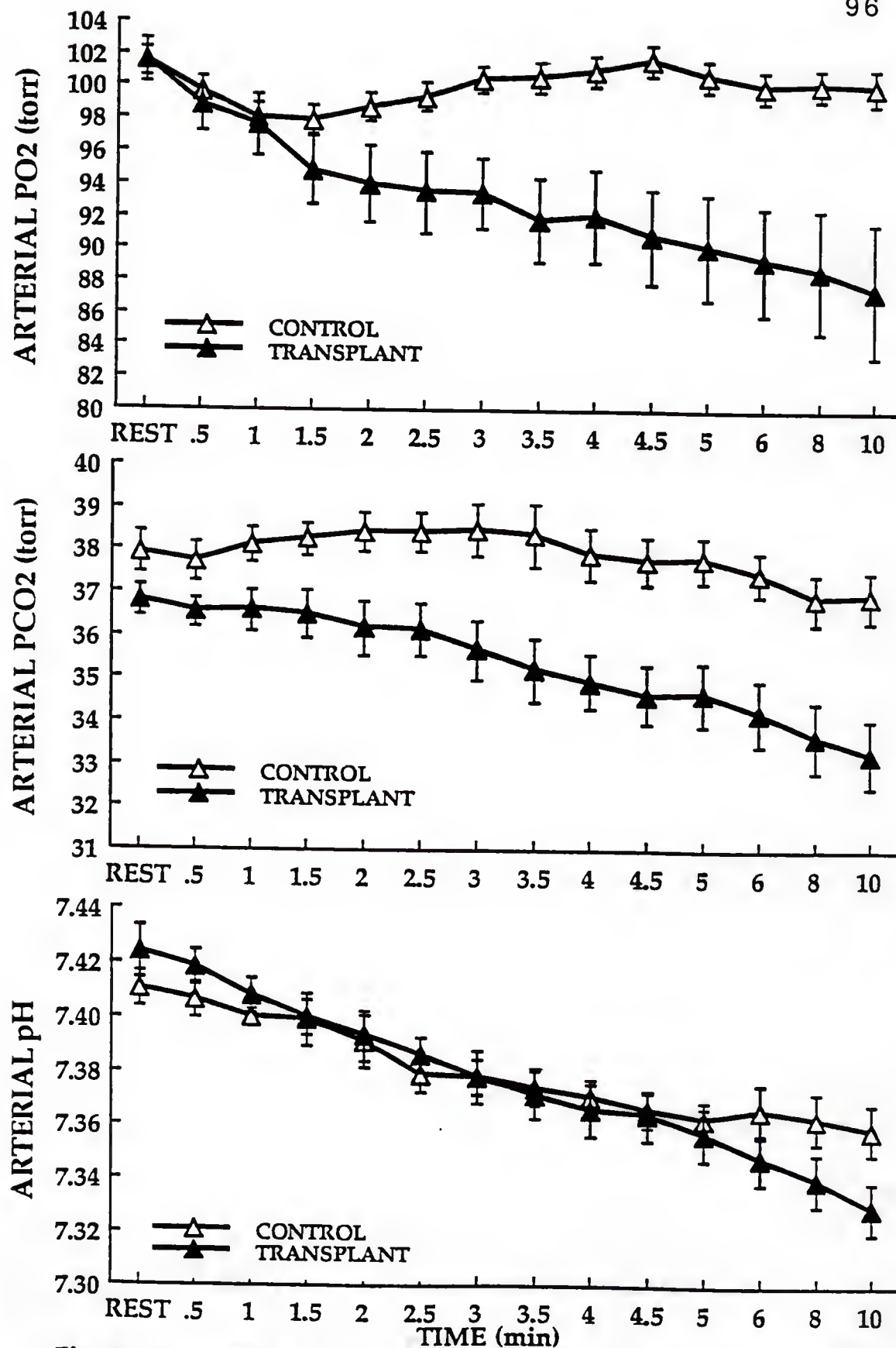


Figure 3. Changes in arterial PO₂, PCO₂ and pH during constant load cycle exercise at 70% of peak power. (n=10 control; n=10 transplant)

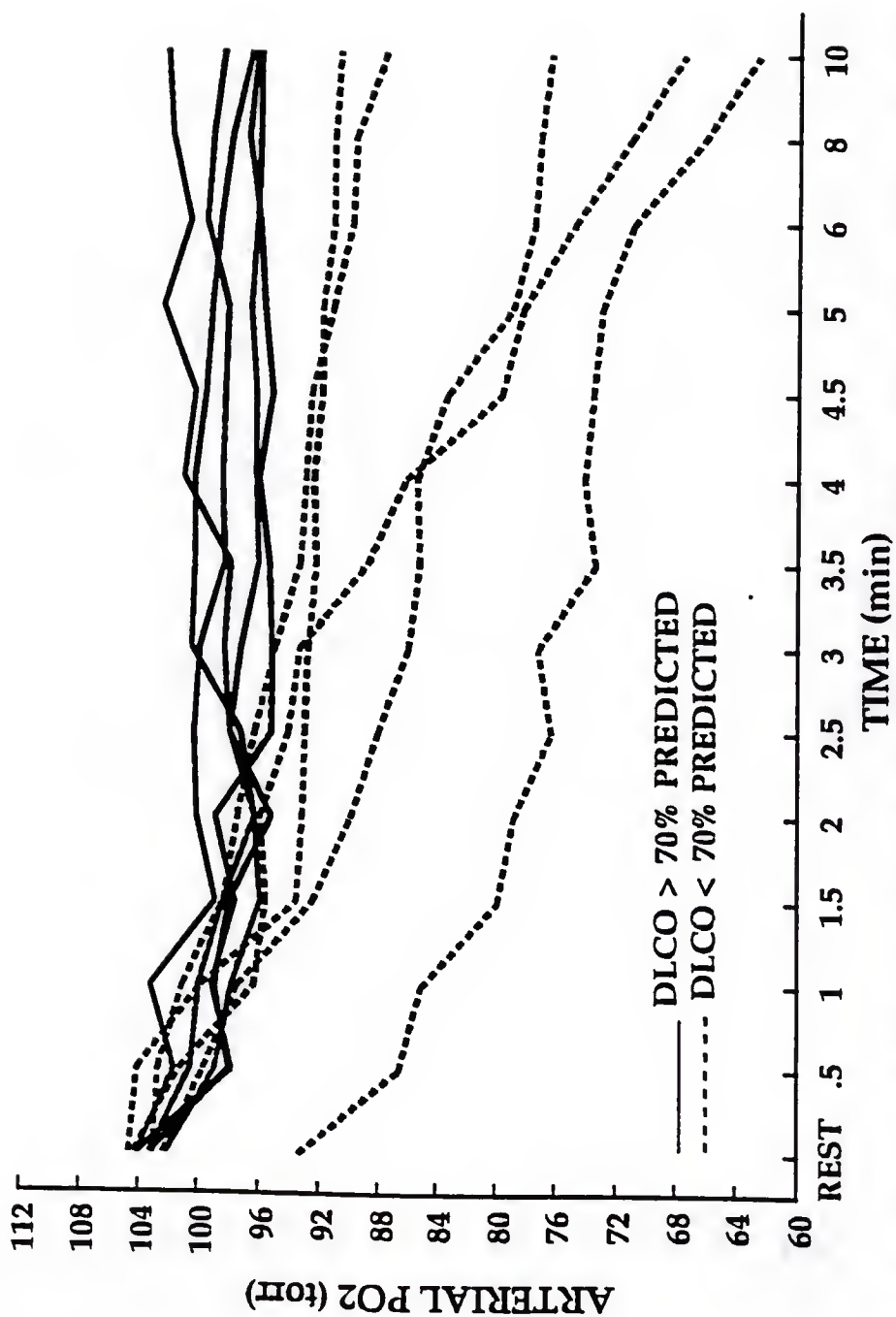


Figure 4. Changes in arterial PO₂ during cycle exercise at 70% of peak power output in transplant patients with normal (n=5) and abnormal (n=5) pulmonary diffusion capacity (DLCO).

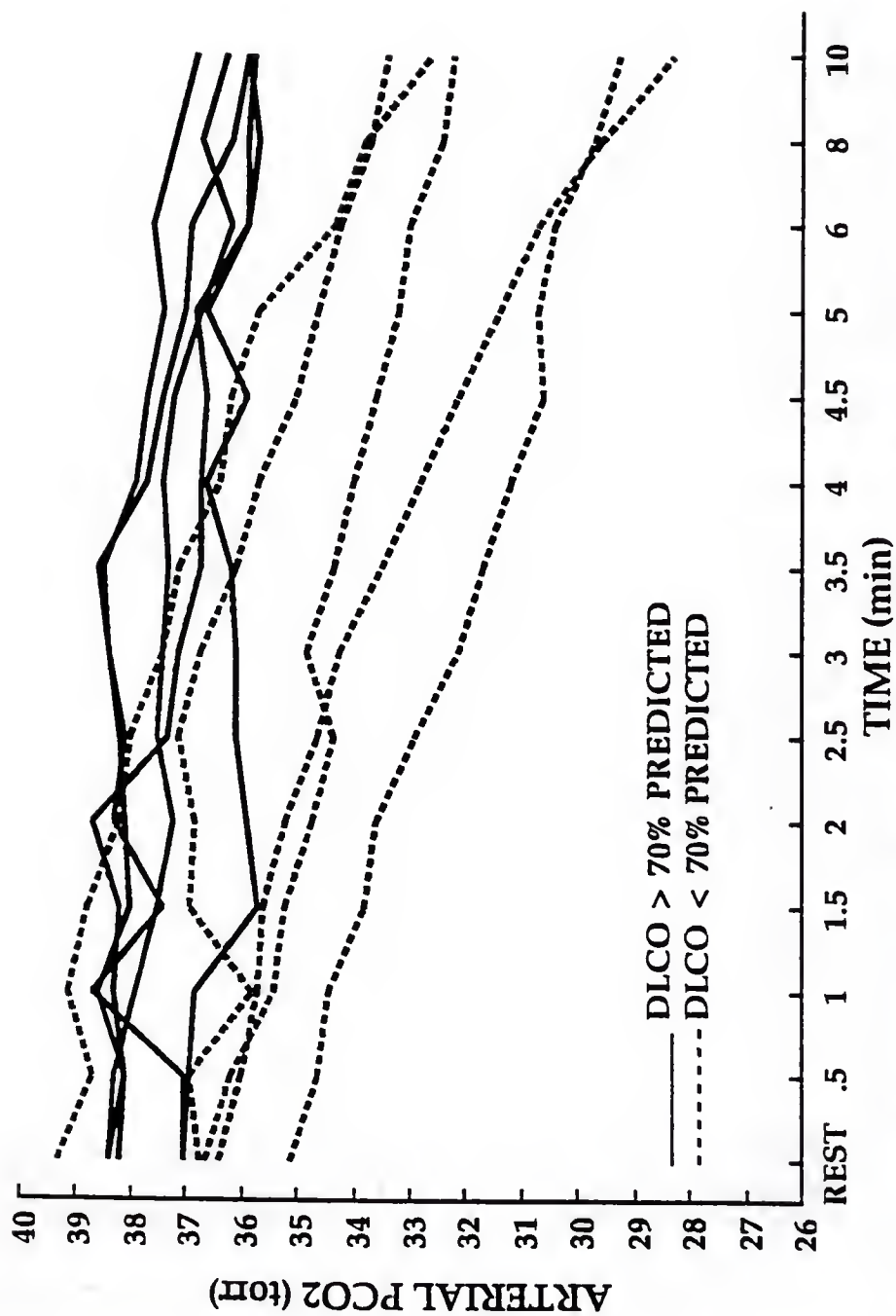


Figure 5. Changes in arterial PCO₂ during cycle exercise at 70% of peak power output in transplant patients with normal (n=5) and abnormal (n=5) pulmonary diffusion capacity (DLCO).

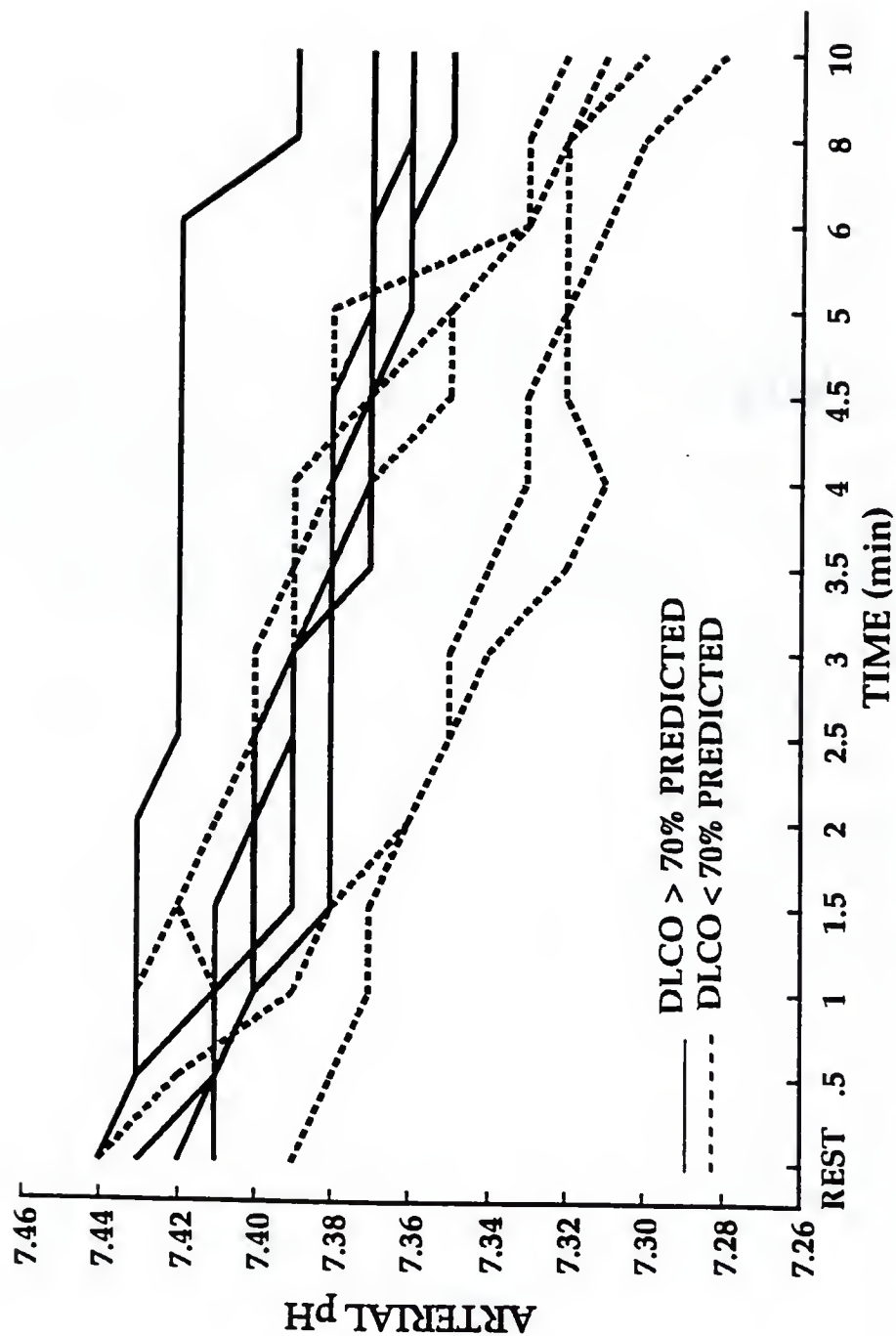


Figure 6. Changes in arterial pH during cycle exercise at 70% of peak power output in transplant patients with normal (n=5) and abnormal (n=5) pulmonary diffusion capacity (DLCO).

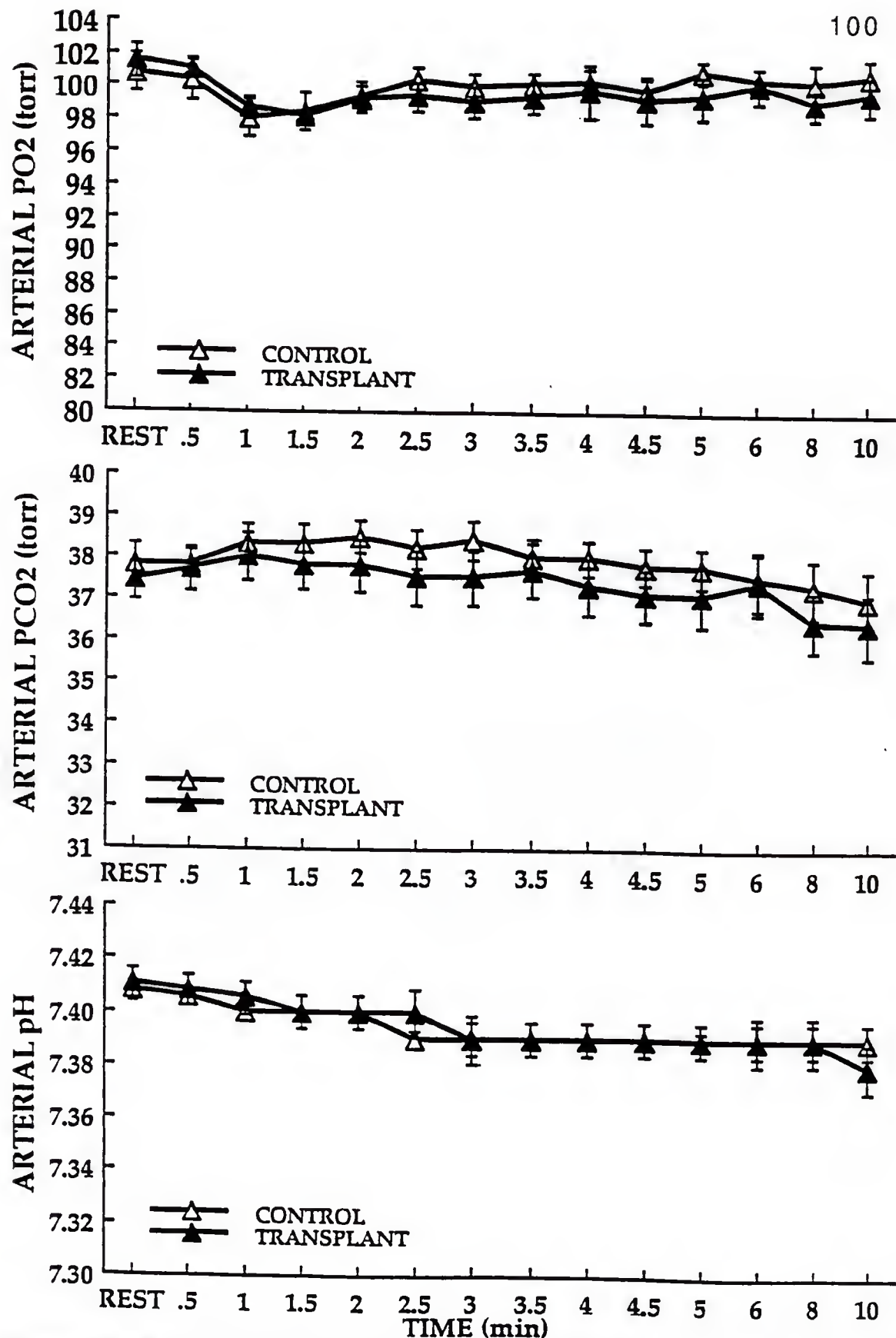


Figure 7. Arterial PO₂, PCO₂ and pH during constant load cycle exercise at 40% of peak power in subjects with normal DLCO. (n=5 control; n=5 transplant)

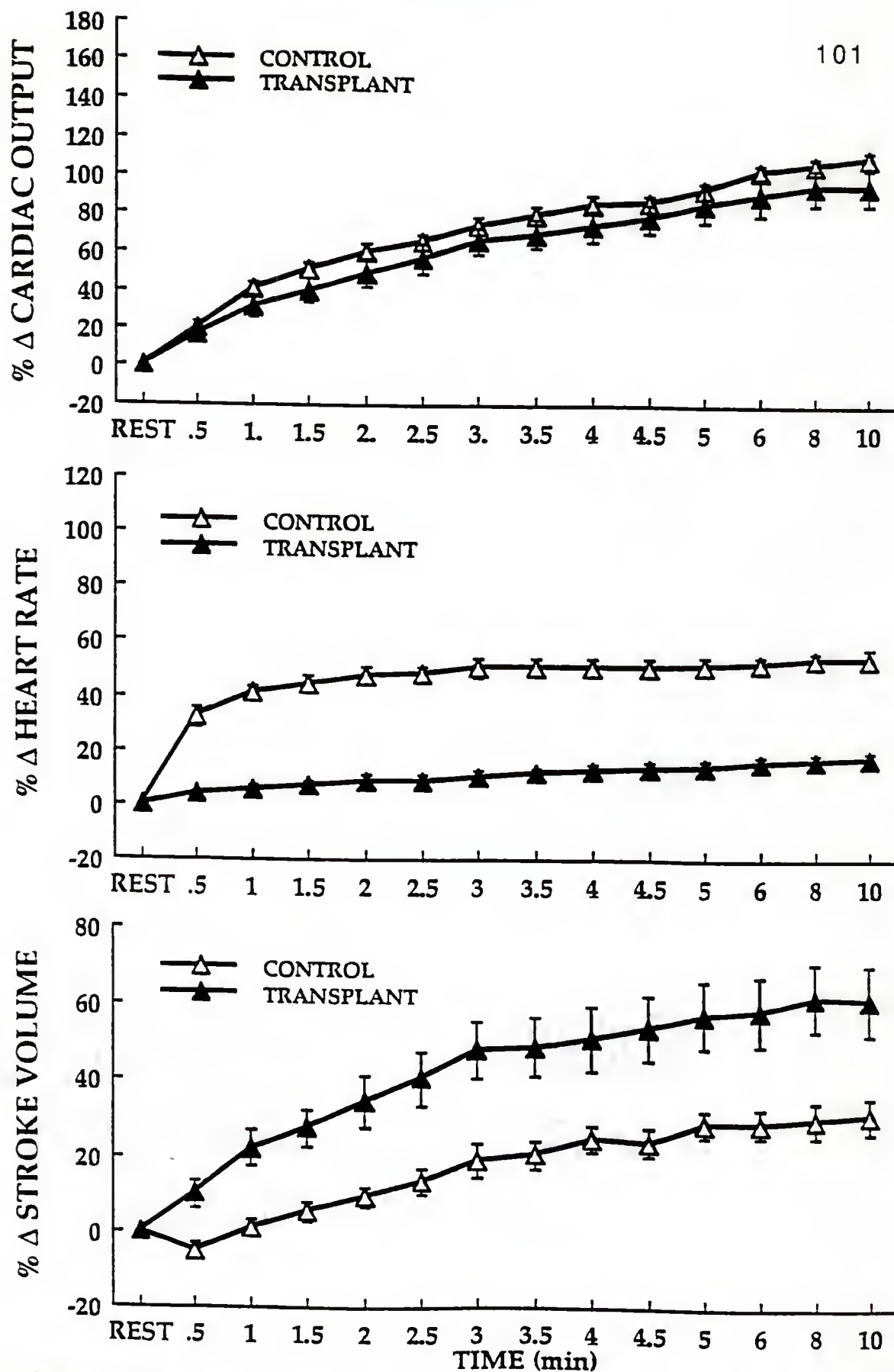


Figure 8 . Relative changes in cardiac output, heart rate and stroke volume during exercise at 40% of peak power. (n=10 control; n=10 transplant)

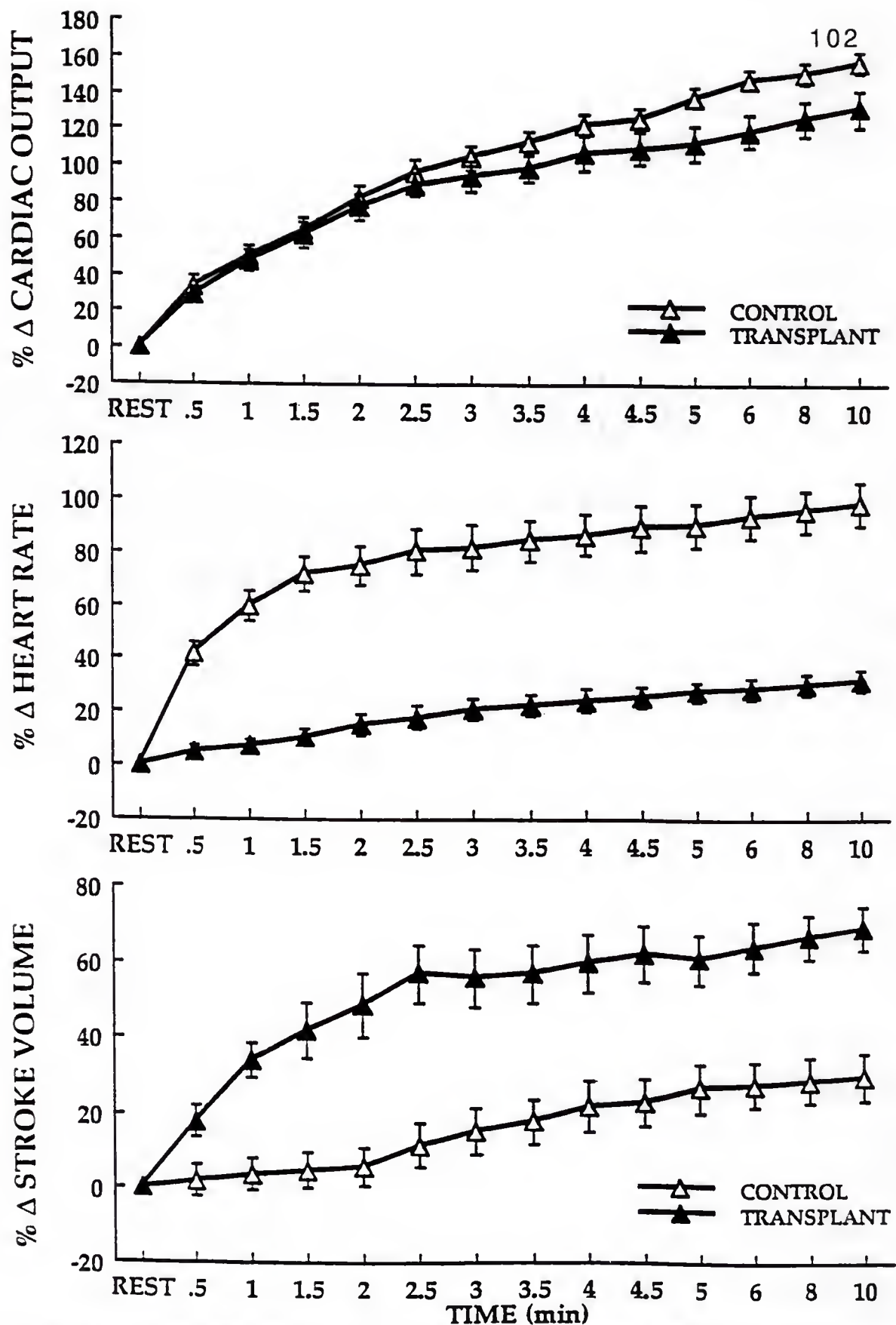


Figure 9. Relative changes in cardiac output, heart rate and stroke volume during exercise at 70% of peak power. (n=10 control; n=10 transplant)

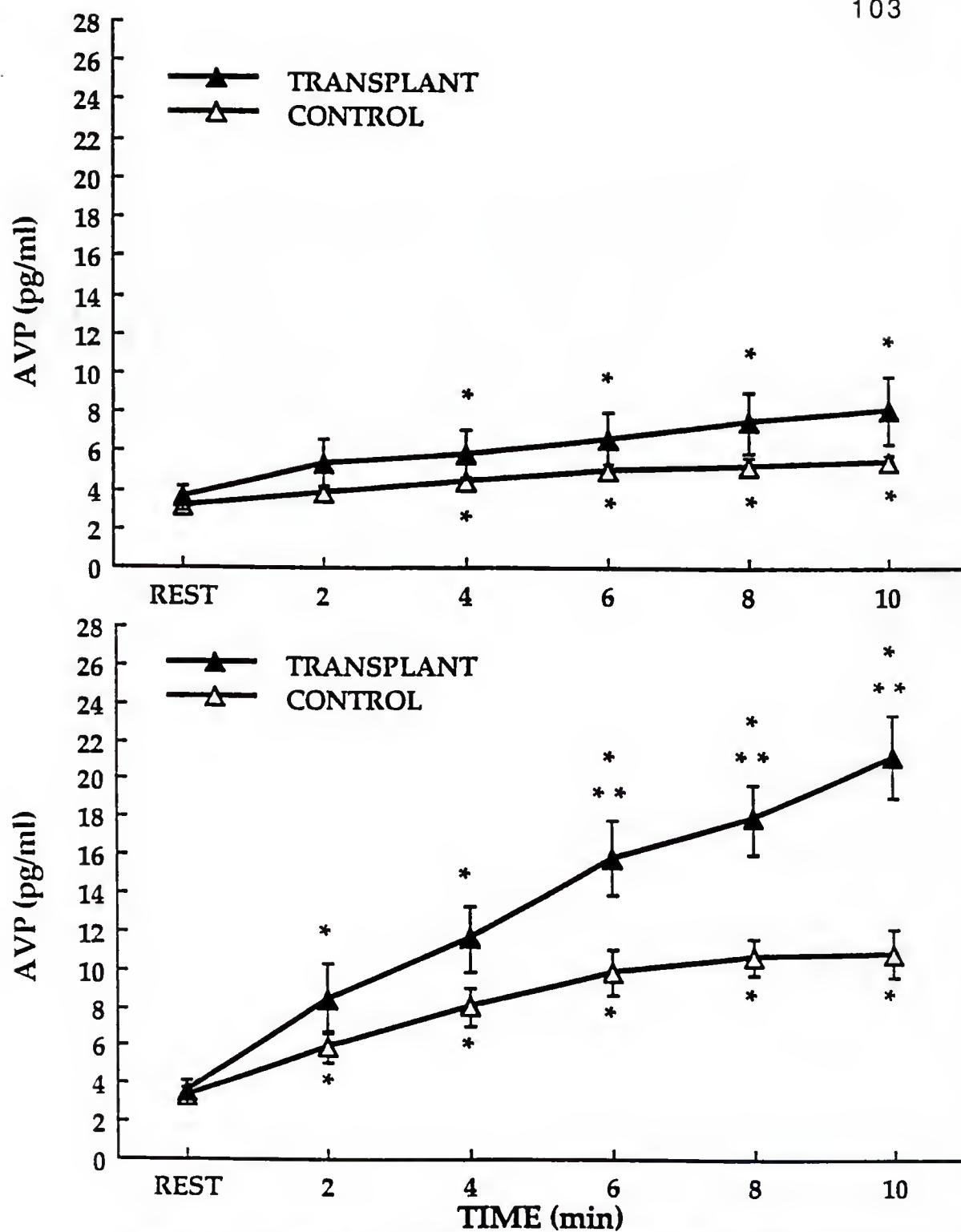


Figure 10. Changes in AVP during constant load cycle exercise at 40% (top) and 70% of peak power output. (mean $\pm$ SEM)

* $p \leq 0.05$ vs rest ** $p \leq 0.05$ transplant vs control

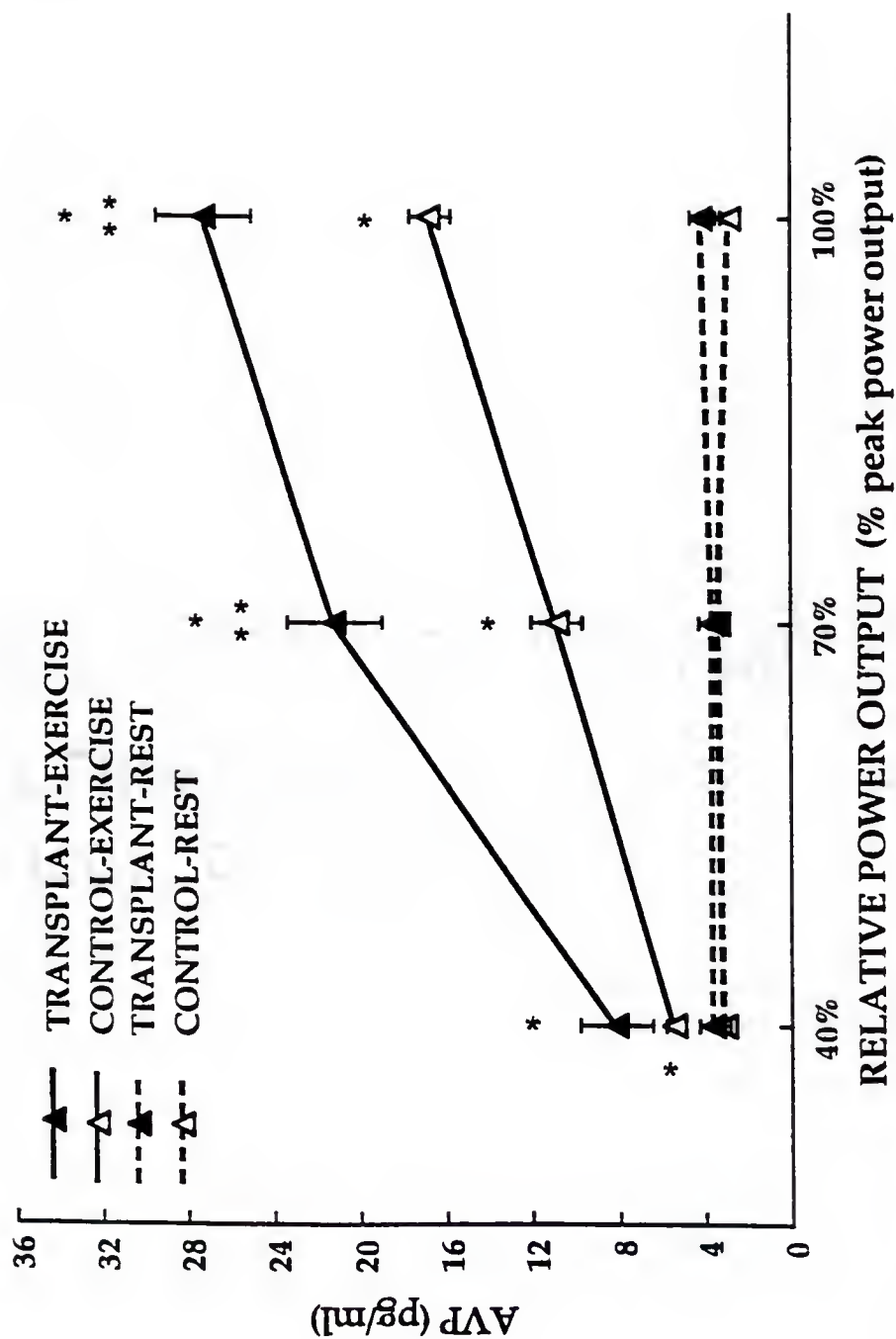


Figure 11. Plasma vasopressin (AVP) at rest and immediately after submaximal and maximal cycle exercise tests. (mean ± SEM) *p ≤ 0.05 vs rest **p ≤ 0.05 transplant vs control

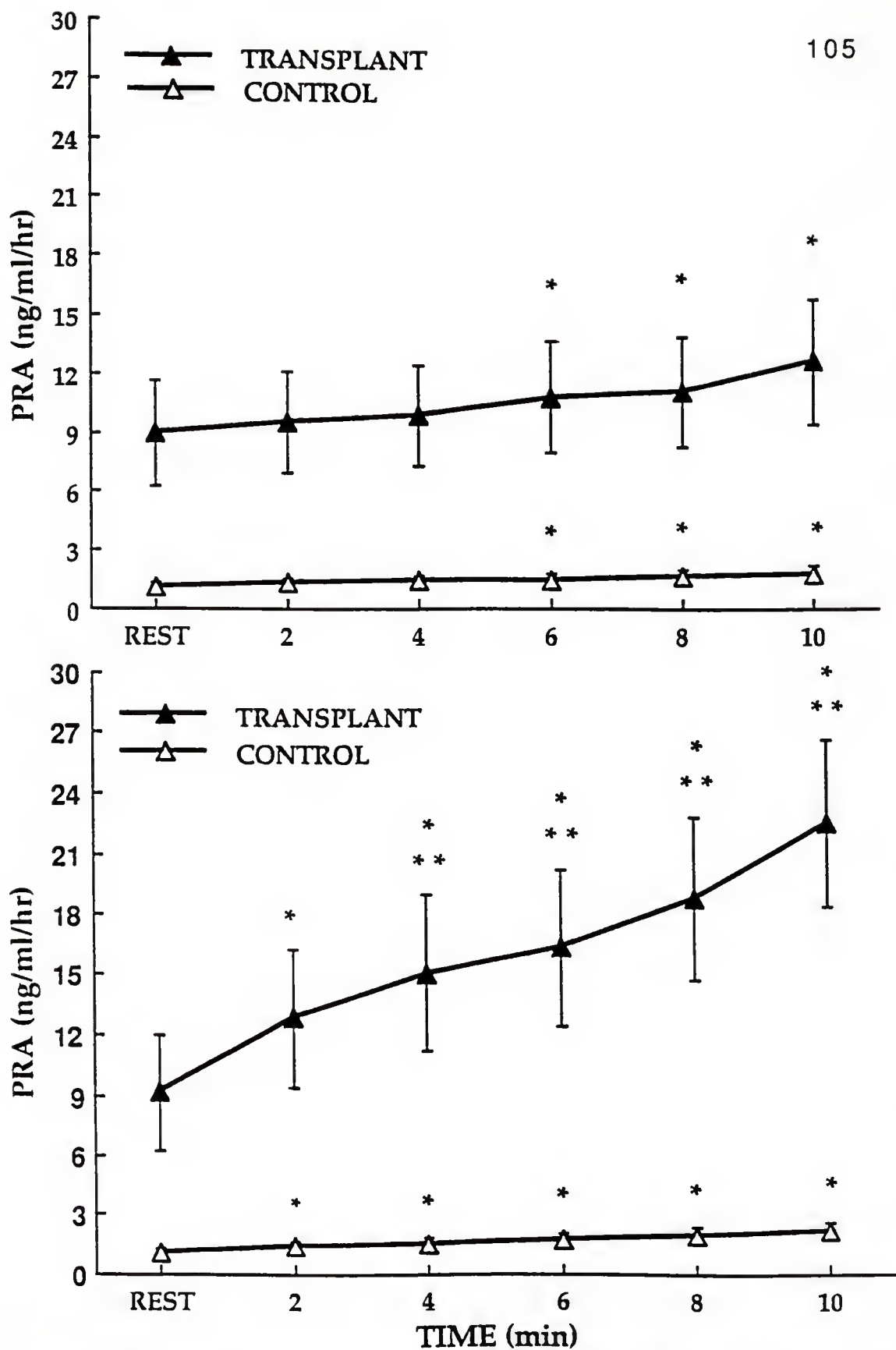


Figure 12. Changes in PRA during constant load cycle exercise at 40% (top) and 70% of peak power output. (mean±SEM)

*p≤0.05 vs rest **p≤0.05 transplant vs control

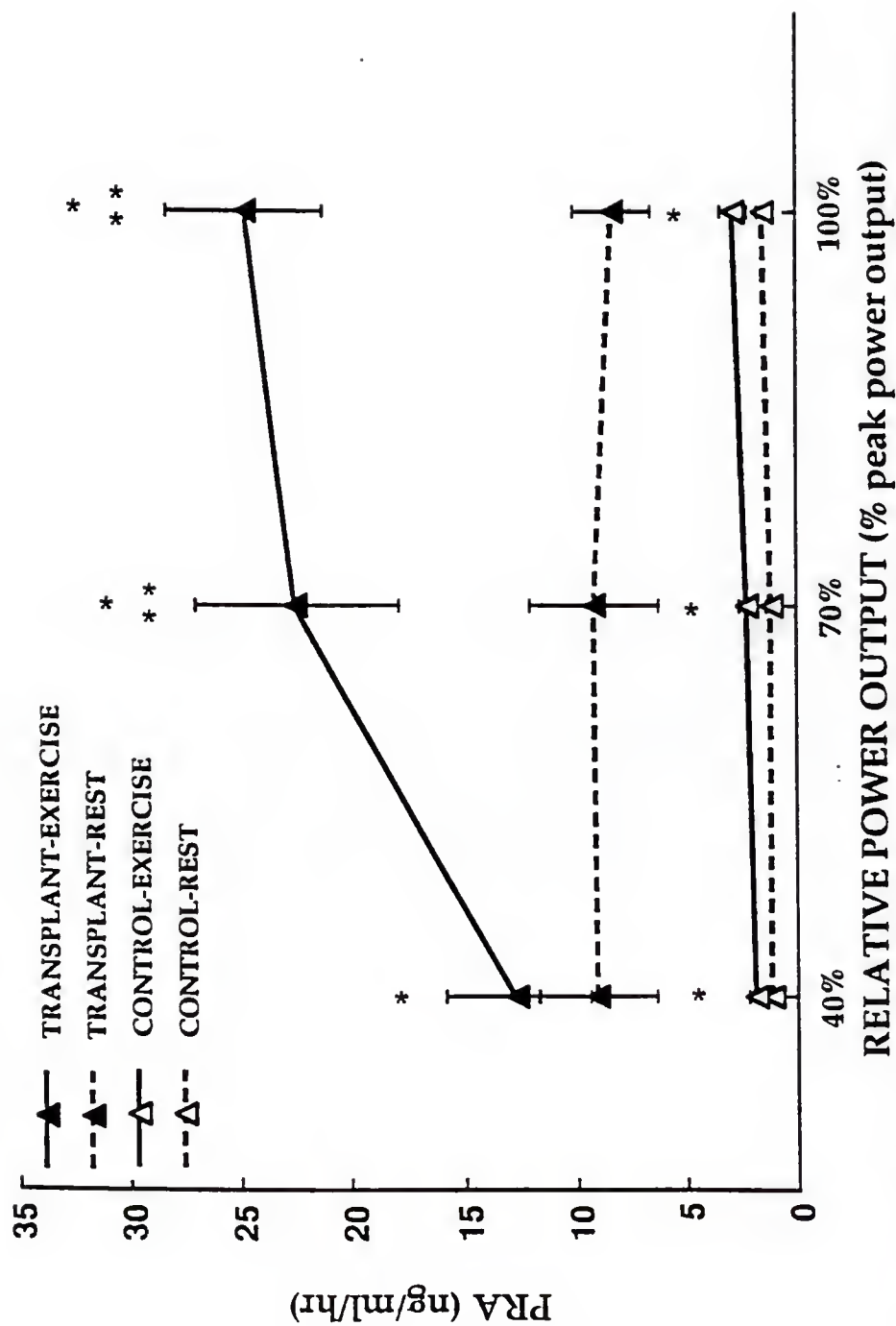


Figure 13. Plasma renin activity (PRA) at rest and immediately after submaximal and maximal cycle exercise tests. (mean $\pm$ SEM) * $p \leq 0.05$ vs rest ** $p \leq 0.05$ transplant vs control

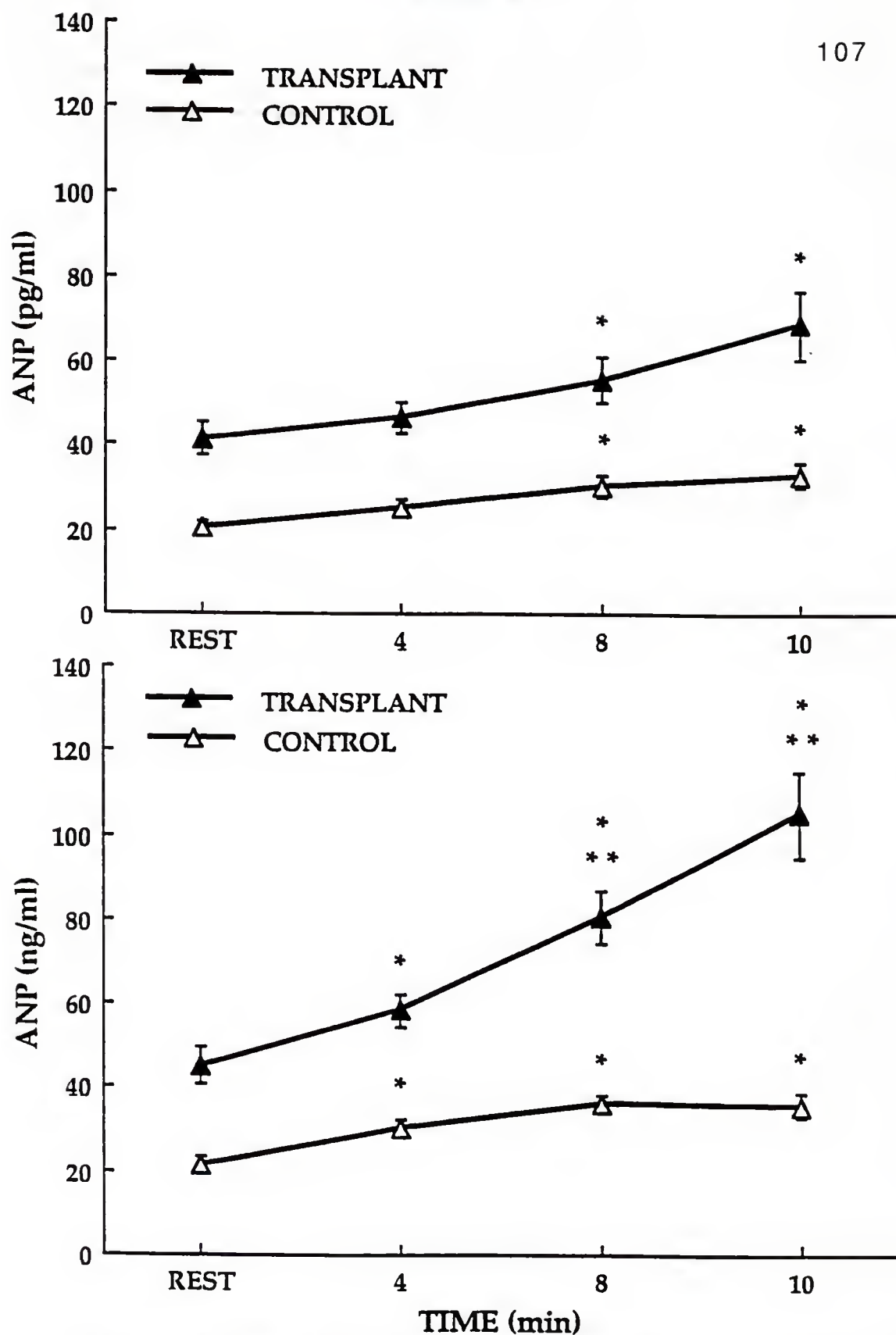


Figure 14. Changes in ANP during constant load cycle exercise at 40% (top) and 70% of peak power output. (mean $\pm$ SEM)

* $p \leq 0.05$ vs rest ** $p \leq 0.05$ transplant vs control

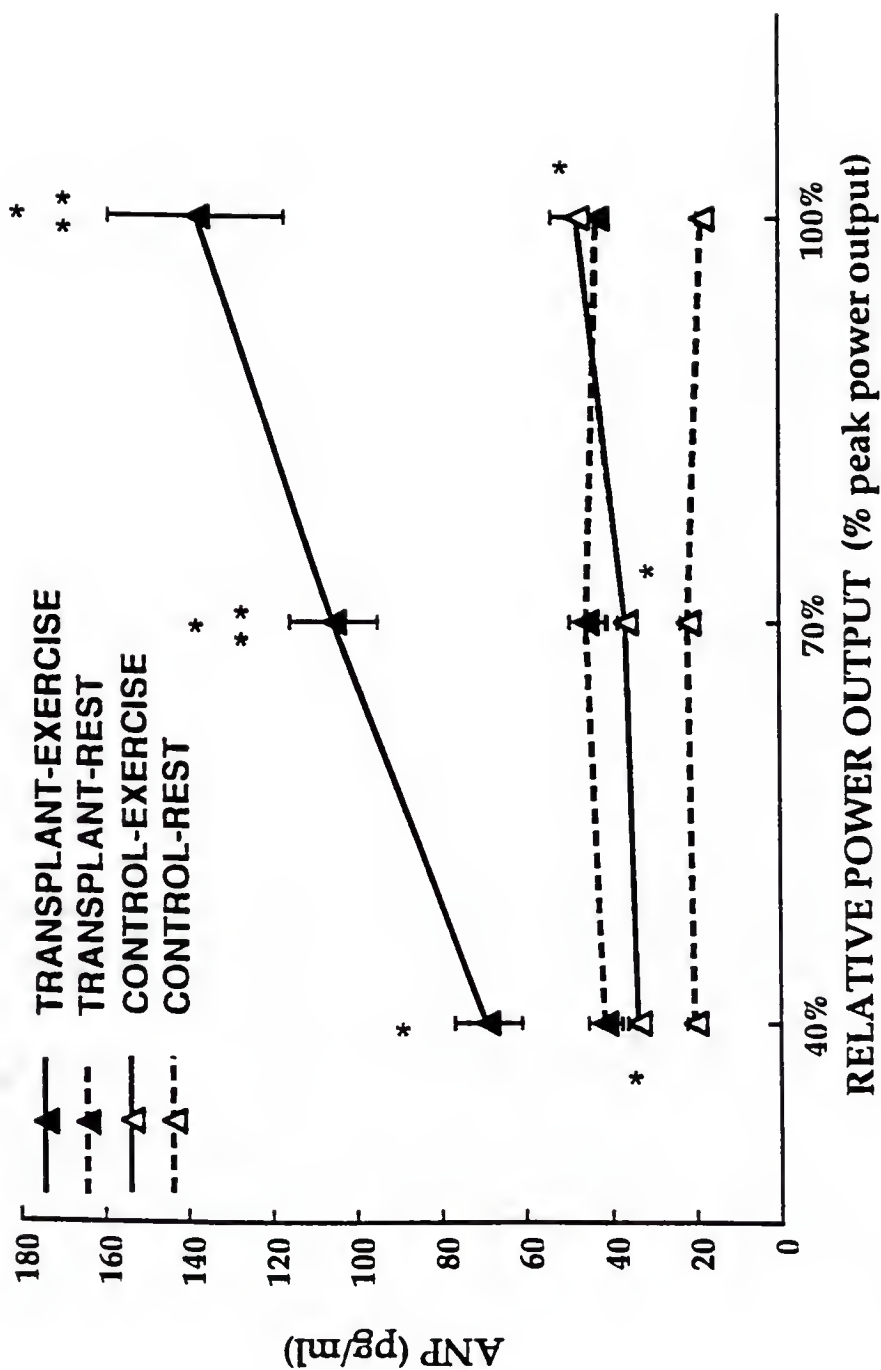


Figure 15. Plasma atrial natriuretic peptide (ANP) at rest and immediately after submaximal and maximal cycle exercise tests. (mean±SEM) * $p \leq 0.05$ vs rest ** $p \leq 0.05$ transplant vs control

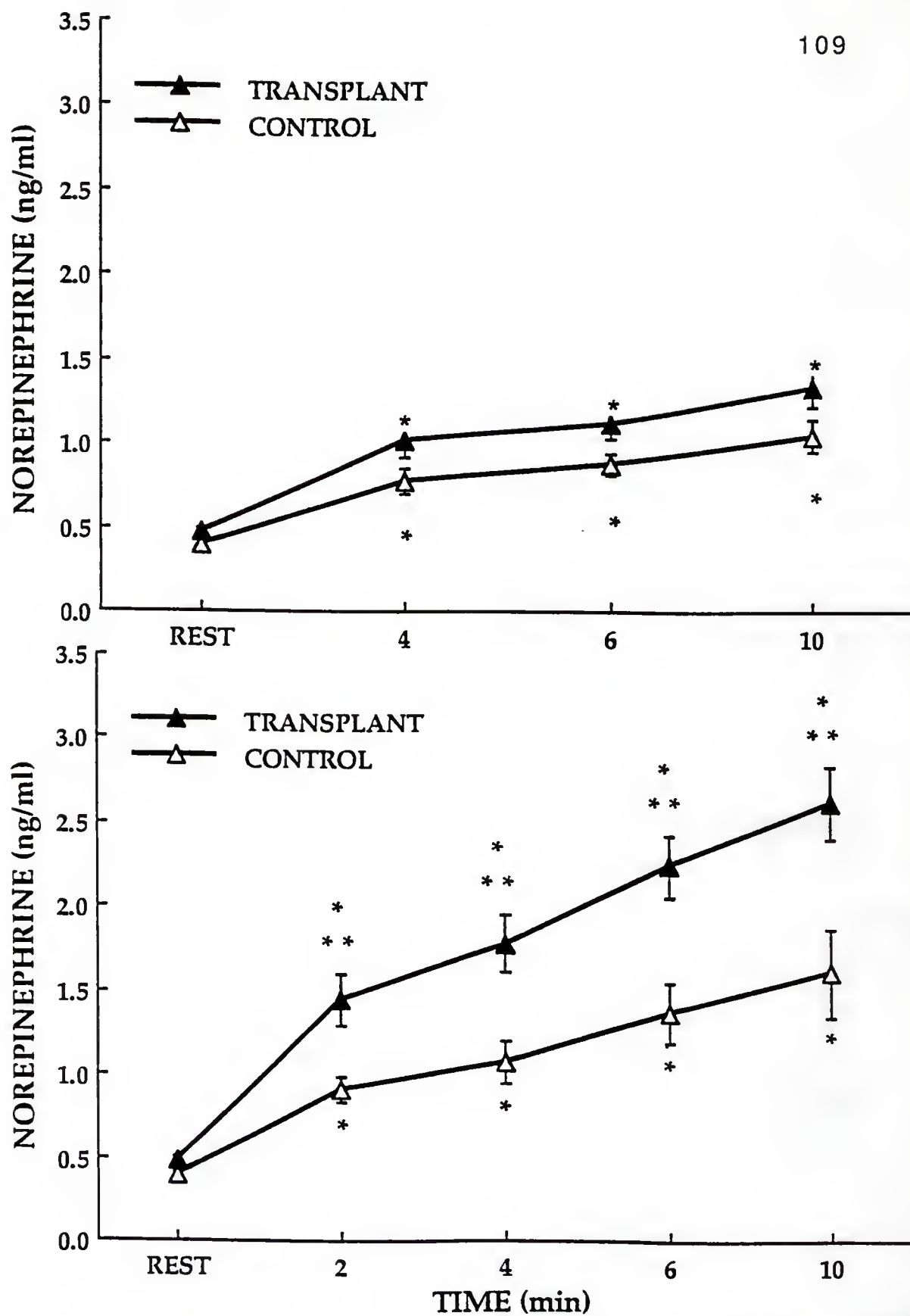


Figure 16. Changes in NE during constant load cycle exercise at 40% (top) and 70% of peak power output. (mean $\pm$ SEM) * $p \leq 0.05$ vs rest ** $p \leq 0.05$ transplant vs control

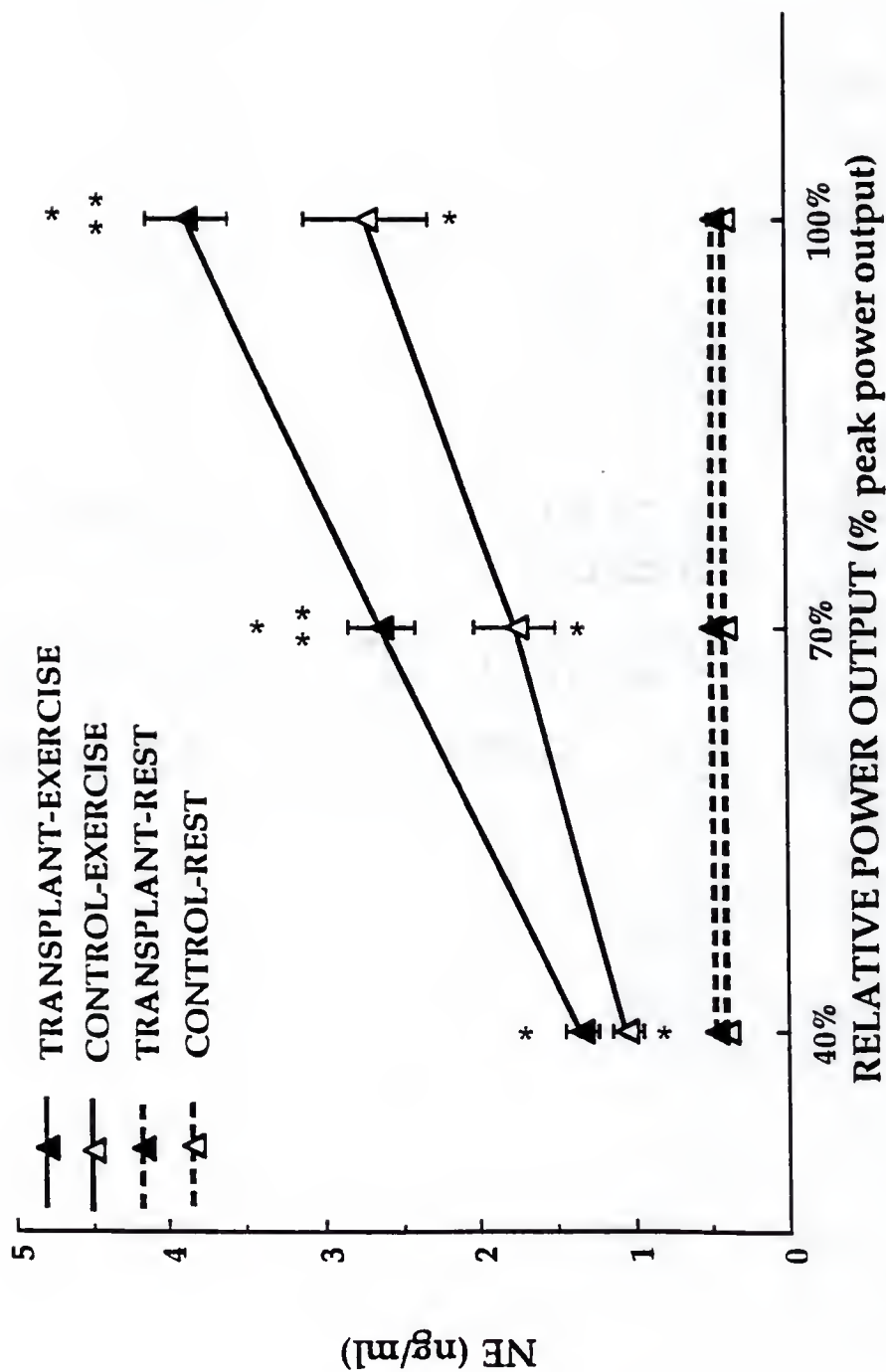


Figure 17. Plasma norepinephrine (NE) at rest and immediately after submaximal and maximal cycle exercise tests. (mean $\pm$ SEM) * $p \leq 0.05$ vs rest ** $p \leq 0.05$ transplant vs control

CHAPTER 5 DISCUSSION

Recent clinical evidence suggests that physiologic mechanisms other than cardiac denervation may be responsible for reduced exercise capacity in HTR. Neuroendocrine abnormalities resulting from cardiac deafferentation, pulmonary gas exchange abnormalities and skeletal muscle weakness are recent postulates for the exercise intolerance. The available data regarding these mechanisms, however, is sparse and conflicting and the physiologic nature of reduced exercise capacity in HTR remains controversial. The present study was designed to answer three questions regarding the etiology of the reduced exercise capacity observed in most HTR: 1) Does Tx alter the neuroendocrine response during exercise? 2) Does Tx and subsequent immunosuppression therapy adversely affect pulmonary function? and, 3) What is the skeletal muscle strength in previously deconditioned HTR placed on chronic corticosteroid therapy? Therefore, the following discussion will be divided into three respective sections.

Neuroendocrine Responses to Exercise

The primary objective of this study was to determine the influence of Tx on the neuroendocrine response to exercise in humans. Our results clearly show that the magnitude of neuroendocrine response to the same relative exercise stress is greater in HTR when compared to innervated

control subjects. The relative increases in AVP, NE, ANP and PRA were significantly greater than the control group at work rates of 70% and 100% of peak power output. This is most likely the result of cardiac deafferentation after Tx, but these data should not be interpreted as having proven this point or eliminated other explanations. There are several possible explanations for these responses considering the fact that neuroendocrine adaptations to exercise and changing plasma volume are complex. The individual roles of multiple hormonal and neural influences are difficult to isolate when, as in the present study, integrated whole-body responses to exercise are determined. With these limitations and complexities in mind, the discussion that follows will address only those neuroendocrine responses to exercise in which the cardiac atria are thought to play a central role: the mechanical release of ANP from atrial myocytes, and the reflex release of AVP, NE and PRA evoked by atrial neural receptors in response to stretch.

Norepinephrine

Elevated concentrations of plasma NE are present in most Tx candidates with congestive heart failure (CHF) (Chidsey et al., 1962; Thomas and Marks, 1978; Goldstein et al., 1981; Colucci et al., 1989; Francis et al., 1990). The increased circulating NE results from increased SNS activity which is characteristic of CHF patients and thought to be a compensatory response for reduced cardiac output. Tx restores cardiac function and reverses CHF (Stinson et al., 1972; McLaughlin et al., 1978; Savin et al., 1980; Pope et al., 1980; Borow et al., 1985a; Pflugfelder et al., 1987), but it is still uncertain whether Tx eliminates or perhaps facilitates the SNS overactivity associated with CHF. More specifically, little is

known regarding the effects of deafferentation on the circulating levels of all pressor hormones during exercise. Others have reported that plasma NE is elevated at the same absolute exercise intensity in HTR when compared to matched controls (Banner et al., 1989; Quigg et al., 1989). It may be inappropriate, however, to conclude from the previous data that SNS overactivity is a consequence of Tx. Considering the fact that peak $\dot{V}O_2$ and peak power output achieved by HTR in those earlier investigations were approximately 60% of that achieved by the control group, each absolute submaximal exercise stage would have required a higher relative percentage of maximal effort in HTR. Therefore, the previously observed increases in plasma NE during graded exercise may only be indicative of the well documented positive linear relationship between relative exercise intensity and SNS responsiveness (Chidsey et al., 1962; Howley, 1976; Goldstein, 1981; Christensen and Galbo., 1983; Rowell and O'Leary, 1990). To our knowledge, this is the first study to compare the SNS response in HTR with age, size and gender matched control subjects during exercise at the same relative exercise intensity.

Basal plasma NE was not significantly different between the two groups (478 vs 394 pg/ml), but HTR demonstrated a trend toward elevated resting NE ($p=0.08$). These results are in agreement with earlier observations that basal plasma NE is slightly elevated in HTR late (>3 months) after Tx (Quigg et al., 1989; Banner et al., 1989; Scherrer et al., 1990; Younis et al., 1990). Exercise at 40% of peak power output evoked comparable increases in plasma NE in each group (figure 17). The relative NE response in HTR during cycle exercise at 70% of peak power output, however, was significantly greater at each measurement period. Plasma

NE levels were also greater in HTR at 100% of peak power output during the SL-GXT. These data indicate that a threshold exercise intensity of >40% of peak power output is required to evoke SNS overactivity in HTR.

Theoretically, the increased levels of plasma NE during exercise at 70 and 100% of peak power output in HTR could reflect either increased release of NE into the circulation or decreased clearance. Although plasma clearance of NE was not assessed, we speculate that clearance abnormalities did not influence the observed plasma NE levels. The fact that, during submaximal exercise testing, plasma NE returned to pre-exercise levels within the 20 minute recovery period between tests at 40 and 70% of peak power output in both groups suggests that NE clearance is normal in HTR without renal dysfunction. The possibility still remains, however, that a decrease in the clearance rate of plasma NE occurs during exercise. Stressors that increase SNS activity tend to diminish renal blood flow and decrease NE clearance from plasma into urine (Dimsdale and Ziegler, 1991).

The elevated NE levels during moderate to strenuous exercise (>40% of peak power output) may be interpreted as humoral compensation for cardiac denervation and reduced cardiac output. Because Tx severs sympathetic nerve fibers and causes total cardiac denervation, the local delivery of NE to the heart by sympathetic nerves is either greatly reduced or entirely absent (Rowan and Billingham, 1988; Regitz et al., 1990). Thus, beta-adrenergic receptor stimulation of the heart is solely due to circulating NE and E. One of the earliest reported studies of the exercise response of the denervated human heart (Pope et al., 1980) observed that increases in heart rate and contractility were highly correlated with

increasing plasma NE levels ($r=0.79$ and $r=0.92$, respectively). Subsequent studies have demonstrated that additional humoral compensation in heart rate reserve is known to occur through hypersensitivity to circulating NE in HTR (Lurie et al., 1983; Vatner et al., 1985; Yusuf et al., 1987; Gilbert et al., 1989; Bristow, 1990; Regitz et al., 1990). Confirmation of this relationship has been shown by others who found that beta blockade has a greater effect on attenuating the chronotropic and inotropic response to exercise in the transplanted human heart compared to the innervated heart (Bexton et al., 1983; Port et al., 1990).

The mechanism of denervation hypersensitivity is currently under debate. Postsynaptic adaptations such as increased beta-receptor density or up-regulation of beta-adrenergic receptor affinity for NE have been proposed (Yusuf et al., 1987; Lurie et al., 1983). Presynaptic changes involving the absence of cardiac neuronal uptake of NE (Vatner et al., 1985; Gilbert et al., 1989) have also been identified as possible mechanisms of supersensitivity to circulating catecholamines. The exaggerated response to circulating NE helps to explain how the exercise capacity of HTR is partially preserved.

Noncardiac factors may also contribute to the increased level of SNS activity observed during exercise in HTR. Scherrer et al. (1990) recently reported that immunosuppression with cyclosporine increases SNS activity. Direct microelectrode recordings of sympathetic nerve activity in HTR treated with cyclosporine revealed that the rate of muscle sympathetic discharge was 2.9 times that in normal controls and 2.7 times that in HTR not receiving cyclosporine. Moreover, plasma NE was 50% higher than in HTR not receiving cyclosporine. Cyclosporine treatment

was also associated with increased SNS activity in myasthenia gravis patients receiving cyclosporine immunosuppression (1.8 times greater than placebo control group). Their results indicate that the SNS excitatory effect of cyclosporine does not depend upon cardiac denervation. However, it is conceivable that cardiac deafferentation and the consequent removal of one source of SNS inhibition may facilitate the excitatory effects of cyclosporine.

Several lines of evidence raise the possibility that peripheral vascular responsiveness may be diminished in HTR. Therefore, greater SNS activity may be required to maintain blood pressure homeostasis during exercise. In congestive heart failure before Tx, maximal skeletal muscle blood flow is reduced during exercise (LeJemtel et al., 1986). The reason for maximal flow limitation has been ascribed by some to impaired cardiac function (Franciosa et al., 1981) and by others to the inability of the peripheral vessels to dilate normally (LeJemtel et al., 1986). Sinoway et al. (1988) used strain gauge plethysmography to measure reactive hyperemic blood flow after arterial occlusion in 10 patients before and after Tx. Maximal peripheral vasodilator capacity remained impaired in some HTR at 4 months postTx although blood pressure and cardiac function had returned to normal. The authors propose that the peripheral vascular system becomes "intrinsically stiff" in response to the low-flow state of congestive heart failure. Peripheral vasoconstrictor responsiveness may also be impaired following CHF. Borow et al. (1985b) studied the sensitivity of the pressor reflex elicited by vascular alpha 1 receptors after Tx. Higher doses (68% > than control) of the alpha 1 agonist methoxamine

were required to produce the same increments in blood pressure (30 to 60 mmHg above baseline) in HTR compared to matched control subjects.

Deafferentation may also be the primary factor promoting SNS overactivity during exercise. Heart rate, cardiac output and arterial pressure increase during exercise and the magnitude of change from baseline is mediated primarily by the vasomotor center of the brain stem (Bevegard and Shepherd, 1967; Christensen and Galbo, 1983; Guyton, 1986). Excitation of the vasomotor center, in turn, is controlled by the hypothalamus and many portions of the cerebral cortex (Guyton, 1986; Rowell, 1986; Rowell and O'Leary, 1990). In opposition to these excitatory mechanisms, impulses from cardiac stretch receptors exert an inhibitory influence on the vasomotor center resulting in reduced sympathetic outflow to the heart, adrenal medulla and vasculature (Karim et al., 1972; Mancina and Donald, 1975; Thames et al., 1978; Thames and Schmid, 1979; Sagawa, 1983). Cardiac stretch receptors are stimulated by increases in filling pressure (preload), afterload and contractility. Since exercise increases preload, afterload and contractility, discharge from cardiac afferents would be increased and the inhibition of sympathetic outflow augmented. It is reasonable to postulate that the inhibitory influence of cardiac afferents is a buffer for the combined excitatory influences of central command and muscle afferent pressor reflexes during exercise. Thus, removal of cardiac baroreflex inhibition through Tx might result in exaggerated neuroendocrine responses to exercise.

Vasopressin

The plasma concentrations of AVP observed at rest (3.6 to 4.2 pg/ml) in our HTR were in agreement with those reported for cardiac denervated

humans (Drieu et al., 1986; Mertes et al., 1991). We are unaware of previous studies that have measured the plasma AVP response to exercise in HTR. Exercise at 40, 70 and 100% of peak power output elicited a dose-response increase in plasma AVP that was significantly greater than baseline for both groups (figure 11). These results concur with the established phenomenon that AVP secretion increases during exercise and the increase is proportional to relative exercise intensity (Van Beaumont et al., 1973; Wade and Claybaugh, 1980; Convertino et al., 1981; Geyssant et al., 1981). The unique finding, however, was that the magnitude of the AVP response to the same relative exercise stress was greater in HTR compared to matched control subjects. Moreover, AVP release may have been even greater if the HTR had not been on prednisone therapy since glucocorticoids inhibit AVP secretion (Raff, 1987).

Factors modulating the release of plasma AVP during exercise are not fully understood. Reduced blood volume during hemorrhage is known to stimulate AVP release via carotid and aortic baroreceptor reflexes (Ledsome and Mason, 1972; Wang et al., 1983; Wang et al., 1988). The well documented acute reduction of plasma volume during exercise has also been identified as one factor responsible for elevated plasma AVP during exercise (Van Beaumont et al., 1973; Convertino et al., 1981; Geyssant et al., 1981). In the present study, plasma volume decreased linearly with increasing exercise intensity (table 7). The plasma volume reductions, expressed as the percent change from baseline, were nearly identical for both groups during each exercise condition. It is unlikely, therefore, that a disproportionate plasma volume reduction is responsible for the AVP response observed in HTR.

Plasma osmolality also mediates AVP release via hypothalamic osmoreceptors. It has been suggested that hyperosmolality caused by transient hemoconcentration, rather than a reduction in plasma volume per se, may be the primary stimulus for vasopressin release during exercise (Convertino et al., 1981). However, available evidence regarding the influence of plasma osmolality on AVP release during exercise is conflicting and suggests that other factors must also be involved. Landgraf et al. (1982) reported that the change in AVP in response to exercise was highly correlated with plasma osmolality. Such a relationship, however, has not been demonstrated by others (Wade and Claybaugh, 1980). Additionally, dehydration or hypertonic saline infusion in resting humans results in 1 to 2 fold elevations in plasma AVP but maximal exercise is known to increase plasma AVP 5 to 10 fold despite the fact that the increase in plasma osmolality may be similar across all conditions (Beardwell et al., 1975; Hammer et al., 1980; Van Beaumont et al., 1973). Although plasma osmolality was not measured in the present study, it seems doubtful that HTR experienced greater hemoconcentration during exercise as evidenced by the fact that relative plasma volume shifts were similar for both groups.

Plasma renin activity was elevated at rest and during exercise in HTR. Plasma renin could thus be an indirect stimulus for AVP release since a relationship between ANG II elevations and increases in plasma AVP have been recorded during exercise (Ramsay et al., 1978; Wade and Claybaugh, 1980; Convertino et al., 1981). ANG II production, however, was inhibited in all HTR with either captopril or enalapril. Basal ANG II and the ANG II increase during maximal exercise were similar in both

groups, suggesting that ANG II was not an important factor mediating the dissimilar AVP response. To a lesser extent, physical pain and anxiety have been shown to function as stimuli for the release of AVP (Schrier et al., 1979). The Borg Scale of Perceived Exertion (RPE) was used in the present study as a subjective index of physical discomfort. The RPE data indicate that the relative level of physical discomfort experienced by transplant and control subjects was similar (table 7). In addition, resting plasma AVP was not statistically different between groups, thus, it is not probable that anxiety could account for the disproportionate rise in plasma AVP during exercise.

In addition to arterial baroreflexes, osmoregulation, ANG II and physical pain, there is a large body of evidence which argues that AVP release from the pituitary gland is modulated by low-pressure stretch receptors in the left atrium (Henry et al., 1956; Gauer and Henry, 1963; Johnson et al., 1969; Ledsome and Mason, 1972; Mancina and Donald, 1975; Quillen and Cowley, 1983). Carotid and aortic baroreceptors participate in the modulation of AVP release under conditions of profound volume or pressure disequilibrium. Hypothalamic osmoreceptors influence AVP release when extracellular body fluid becomes highly concentrated. Relatively minor shifts in left atrial pressure, however, elicit marked alterations of plasma AVP (Quillen and Cowley, 1983). Quillen and Cowley (1983) demonstrated that changes in left atrial pressure of ± 3.5 mmHg in dogs resulted in 100% alterations of plasma AVP concentrations. The sensitivity of this reflex in primates has been challenged (Gilmore and Zucker, 1978) but the available evidence indicates that the reflex is important in larger upright primates and humans.

Support for this conclusion is provided by the observation that the AVP response to volume depletion is abolished in cardiac denervated HTR (Drieu et al., 1986). Drieu et al. (1986) found that the AVP response to volume depletion with frusemide (40mg) was negligible in 13 HTR (AVP=4.5 and 4.7 pg/ml before and after lasix) but plasma AVP increased 45% from baseline in the control group. These results are consistent with findings after volume depletion and atrial stretch-receptor unloading via hemorrhage in cardiac denervated dogs (Wang et al., 1983; Wang et al., 1988). In a series of experiments, Wang and coworkers (1983; 1988) established that mild hemorrhage (10 ml blood/Kg body weight) caused significant increases in plasma AVP in control dogs but not in cardiac denervated dogs. During hemorrhage of 20 and 30 ml of blood/kg of body weight, the plasma AVP increase in cardiac denervated dogs was only 10% of that observed in the control dogs (33 vs 300 pg/ml).

The effects of removing the inhibition to AVP release during atrial stretch-receptor loading has also been demonstrated in cardiac denervated dogs (Thames and Schmid, 1979; Goetz et al., 1981; Fater et al., 1982). Fater et al. (1982) observed that plasma AVP concentrations decreased from 4.4 to 2.6 pg/ml after 25 minutes of balloon inflation in sham-operated dogs but plasma AVP was not affected by inflation of a balloon in the left atrium of cardiac denervated dogs. Similarly, Thames and Schmid (1979) observed that denervation of cardiac vagal afferent nerves in dogs resulted in a 10 fold elevation of plasma AVP and a corresponding antidiuresis during left atrial distension, whereas denervation of the carotid and aortic baroreceptors failed to cause a release of AVP. Goetz et al. (1981) anesthetized the left atrial receptors in conscious dogs and, consistent with

the decreased level of receptor activity, noted reduced urine and sodium excretion.

In summary, we are unaware of any data available on the modification of the AVP response to exercise in cardiac denervated humans. Nonetheless, it seems reasonable to postulate that the disproportionate elevation in plasma AVP during exercise at 70 and 100% of peak power can be attributed to cardiac deafferentation. Upright exercise in humans increases cardiac preload and presumably atrial stretch. It is conceivable that the inhibitory influence on AVP secretion evoked by stretch of atrial receptors during exercise exerts a buffering influence upon the factors described above that promote AVP release. Removal of the inhibitory atrial influence through cardiac deafferentation would eliminate this buffer and leave unopposed those factors that potentiate increased AVP secretion.

Renin-Angiotensin-Aldosterone-System

Activation of the RAAS is common in CHF patients (Francis et al., 1990). Angiotensin converting enzyme (ACE) inhibitors decrease RAAS activity and improve survival in patients with CHF (Consensus, 1987), suggesting that the RAAS plays an important role in CHF pathology. It is unclear, however, whether RAAS activation continues postoperatively in HTR who no longer have severe left ventricular dysfunction. Reports of increased (Schuler et al., 1987), decreased (Held et al., 1989) and normal (Bellet et al., 1985; Dussaule et al., 1990) basal PRA in HTR not receiving antihypertensives have appeared in the related literature.

Baseline PRA was elevated in all HTR in the present study (figure 13). However, all HTR were being treated for mild-to-moderate hypertension

and the antihypertensive medications were not withheld during the study. Four of the HTR were receiving furosemide, and PRA is known to increase in HTR after plasma volume depletion by lasix (Drieu et al., 1986). All eleven HTR were also receiving ACE inhibitors (three HTR on captopril; eight HTR on enalapril). ACE antagonists increase PRA (ie. angiotensin I) by blocking the conversion to ANG II and thereby opening the RAAS feedback loop (Swedberg et al., 1990). Additionally, all HTR were immunosuppressed with cyclosporine, which has been shown to be a stimulus for increased PRA (Schuler et al., 1987).

We also found that the relative increase ($\% \Delta$ from baseline) in PRA in HTR during strenuous exercise is greater than in normal control subjects. Although elevated basal PRA in our HTR may be explained by the medication regimen, the explanation for the observed PRA stimulation during exercise is less clear. Of the several mechanisms known to promote secretion of renin (decreased renal perfusion pressure; reduced sodium in distal tubules; SNS stimulation), change in the activity of the renal sympathetic nerves appears to be the primary stimulus. Electrical stimulation of renal sympathetic nerves at intensities that do not alter renal blood flow or perfusion pressure results in an increase in renin secretion (LaGrange et al., 1973). Renal sympathetic nerves have been shown to be the principal mediators of the increase in renin secretion during electrical stimulation of the vasomotor center and during orthostasis (Zanchetti and Stella, 1975).

Exercise also produces significant elevations in plasma epinephrine (E) and plasma E has been shown to be associated with renin release (Wade and Claybaugh, 1980; Convertino et al., 1981). Beta blockade of

circulating catecholamines attenuates the renin response to exercise in humans, further supporting the relationship between humoral factors and PRA stimulation (Lijnen et al., 1979). However, beta-blockade also decreases mean arterial pressure and renal perfusion pressure. Consequently, it is difficult to determine whether plasma E directly stimulates renin or indirectly influences renin release via alterations in renal hemodynamics. Zambraski et al. (1984) blocked renal sympathetic nerve traffic with guanethidine and found PRA to be decreased 41 and 73% at rest and during heavy exercise, respectively. Because guanethidine blocks sympathetic nerve traffic but does not interfere with the responses to circulating E, their data imply that the increase in PRA with exercise is mediated primarily by stimulation of the renal nerve rather than elevated plasma E.

Thus, the available data implies that any receptor system that can change renal sympathetic nerve activity reflexly can influence the secretion of renin during exercise in humans. Numerous studies with dogs have demonstrated that atrial stretch receptors influence renin secretion via vagal afferents that exert a tonic inhibition upon the vasomotor center and sympathetic nerve traffic to the kidney (Karim et al., 1972; Mancina and Donald, 1975; Thames et al., 1978; ; Goetz et al., 1981; Fater et al., 1982). Karim and colleagues (1972) demonstrated that left atrial distention by balloon inflation produced an instantaneous decrease in efferent renal nerve activity to 37% of that in the controls.

Fater et al. (1982) showed that the normal reduction in PRA did not occur during 25 minutes of left atrial distension by balloon inflation in cardiac denervated dogs. Myers and coworkers (1988) recently tested atrial

stretch reflex responsiveness in human HTR. PRA in HTR was elevated 3 fold higher than normal controls before, during and after central volume expansion by water immersion. The findings in the present study further support the hypothesis that cardiac denervation in humans may result in unopposed efferent sympathetic nervous traffic to the kidney.

Atrial Natriuretic Peptide

Resting plasma ANP in HTR was 2.4 times greater than in the control group. The finding of elevated resting ANP is consistent with others who have measured baseline ANP in this population (Magovern et al., 1987; Deray et al., 1988; Singer et al., 1990; Mertes et al., 1991). The ANP response to exercise at 40% of peak power output was similar between groups, but during exercise at 70 and 100% of peak power output, both absolute and relative increases in ANP were greater in HTR (figure 15).

Plasma ANP responses during graded submaximal exercise have not been previously reported in HTR. Singer et al. (1990) found that plasma ANP levels at maximal exercise were elevated in HTR when compared to innervated matched controls (204 vs 151% increase from rest), but the difference between groups was not statistically significant. The apparent discrepancy between our findings and those of Singer et al. (1990) could be explained, at least in part, by the fact that more atrial tissue was present in our HTR. Their surgical procedure reportedly increased atrial tissue by approximately 25%. The surgical technique used with all HTR in the present study resulted in the approximate doubling of right and left atrial mass (Baumgartner et al., 1990). The native heart is sectioned at the level of the atrio-ventricular valves and nearly the entire donor heart is anastomosed, so that there is 90 to 100% more atrial tissue in the heart

after Tx. The increased atrial tissue, with increased secretory ANP granules could be expected to contribute to the elevated plasma ANP concentrations that were observed.

Although increased atrial tissue mass is one possible factor, the mechanism(s) responsible for elevated plasma ANP at rest and during exercise in HTR remain unclear. Early studies indicated that elevated atrial pressure, specifically atrial stretch due to central hypervolemia was the primary stimulus for ANP release (de Bold et al., 1981; Raine et al., 1986). Although absolute plasma volume was not measured in the present study, volume expansion (15%) and increased resting atrial pressure have been observed in HTR and thus may influence ANP release (Bellet et al., 1985; Greenberg et al., 1985; Pflugfelder et al., 1988; Rudas et al., 1990; Hosenpud et al., 1990). Left ventricular end diastolic volume during exercise is also known to increase much more than normal in HTR and may influence supraventricular pressures (Stinson et al., 1972; McLaughlin et al., 1978; Pflugfelder et al., 1987; Pflugfelder et al., 1988; Younis et al., 1990; Rudas et al., 1990).

During exercise at 70 and 100% of peak power output, heart rate increased 33 and 57%, and 98 and 149% in HTR and control groups, respectively. The greater relative increase in ANP at the same relative exercise intensity may indicate that atrial pressure and thus atrial wall stretch is greater during exercise when the heart rate response is blunted by denervation. It is also reasonable to postulate that because of the larger atrium after Tx, higher atrial wall tension may occur at a given atrial pressure as a result of Laplace's Law (Guyton, 1986). Unfortunately,

however, invasive atrial pressure measurements are difficult to obtain during upright exercise and these data are not available in HTR.

Hypertension has also been associated with elevations in ANP, especially when the hypertension is severe. Arendt et al. (1986) found baseline ANP to be elevated 7 fold in a group of 27 hypertensive patients when compared to a normotensive control group (ANP=62.2 vs 8.8 pg/ml, respectively). Resting blood pressure in our HTR, however, was well controlled (group mean= 126/90 mmHg) with only mild elevations of diastolic pressure in five HTR. The increase in systolic blood pressure during exercise was significantly less in HTR compared to the control group. Furthermore, elevated plasma ANP has been found in HTR in the absence of increased right atrial pressure (Magovern et al., 1987; Dussaule et al., 1990) or high blood pressure (Deray et al., 1988).

Renal dysfunction has also been associated with elevated plasma ANP (Pruszczyński et al., 1987). Cyclosporine immunosuppression therapy in heart as well as kidney transplantation is frequently complicated by the development of renal toxicity and dysfunction (Myers et al., 1984; Taube et al., 1985; Humes et al., 1985; Racusen et al., 1986; Macris et al., 1989). None of the HTR in the present study, however, were experiencing significant renal dysfunction.

Corticosteroid therapy, ie. prednisone, could be another factor responsible for elevated plasma ANP in HTR. Gardner et al. (1988) recently demonstrated (in vitro) stimulation of ANP gene expression in rat cardiac myocytes through corticosteroid treatment. Furthermore, prednisone is known to promote water and sodium retention by the kidney and may thus indirectly cause elevated plasma ANP through

volume expansion. It is unclear, however, how prednisone stimulation of ANP gene expression could be responsible for the significantly greater relative increase in plasma ANP in HTR during a brief bout of exercise. There is also evidence to suggest that the general level of sympathetic overactivity observed in our HTR could be a stimulus for increased ANP release during exercise. Infusions of pressor hormones such as ANG II and NE have evoked pronounced increases in plasma ANP in humans (Uehlinger et al., 1986).

It is also possible that ANP release from atrial myocytes is under neural control. If ANP release was tonically inhibited, the cardiac denervation associated with Tx might be expected to result in unopposed release of ANP. The observed exaggerated ANP response to a comparable exercise stimulus may thus be related to cardiac denervation. Pruszczyński et al. (1987) performed stepwise decremental and incremental changes in plasma volume in five normally innervated and five cardiac denervated renal failure patients. Plasma ANP concentrations increased during volume expansion in both groups but the ANP response was greater in both absolute and relative terms in the cardiac denervated group. Further research, however, is necessary to explore the possible effects of cardiac denervation on ANP secretion in humans.

Neuroendocrine Summary

Resting plasma ANP and PRA were significantly ($p \leq 0.05$) elevated in HTR. Plasma NE and AVP concentrations were also elevated in HTR compared to the control group but the difference did not reach statistical significance. Plasma NE, AVP, PRA and ANP responses to the same relative exercise stress were greater in HTR when compared to innervated

control subjects. However, a threshold exercise intensity of >40% of peak power output is required to evoke the heightened responses. The observed neuroendocrine overactivity may be ascribed to cyclosporine immunosuppression, antihypertensive medication, electrolyte imbalance or SNS compensation for decreased cardiac output.

However, an alternative and attractive explanation for stimulation of NE, AVP, PRA and perhaps even ANP release during exercise is that Tx results in cardiac deafferentation. Virtually all of the neuroendocrine responses to exercise in HTR could be explained by ablation of atrial stretch receptor afferents. That SNS activity and reflex secretion of AVP and PRA might be largely unopposed is certainly plausible, given the ablation of afferent neural connections between the donor heart and brain of these subjects.

Orthotopic Tx severs all of the nerves projecting to and from the donor heart. In animal models, limited reinnervation by the sympathetic nervous system usually occurs within the first year after either homotopic or autotopic Tx (Willman et al., 1962; Willman et al., 1964; Peiss et al., 1966; Kontos et al., 1970; Mohanty et al., 1986a). Microscopy studies have documented regrowth of sympathetic nerves over the suture line between the donor heart and recipient atria (Peiss et al., 1966). Physiologic studies have demonstrated that heart rate and contractility can be increased by reflex sympathetic maneuvers or direct neural stimulation in dogs (Peiss et al., 1966; Kontos et al., 1970).

In contrast to the evidence for sympathetic reinnervation in animals, evidence for reinnervation after Tx in humans is lacking. Reflex changes in heart rate that normally occur during central volume loading by

passive leg elevation (Pope et al., 1980; Pflugfelder et al., 1988), at the initiation of exercise or during the valsalva maneuver and orthostasis are absent (Schroeder, 1979; Savin et al., 1980; Pflugfelder et al., 1987; Nixon et al., 1989; Colucci et al., 1989; Quigg et al., 1989; Scherrer et al., 1990). Moreover, no histologic evidence of reinnervation has been found in endomyocardial biopsy samples obtained from HTR up to 12 years after transplantation (Rowan et al., 1988; Regitz et al., 1990). The available evidence argues that the neural link between stretch receptors in the donor heart and the central nervous system remains disconnected indefinitely in humans.

Mechanoreceptors in the residual native atria may also be rendered unable to contribute to the tonic inhibition of AVP, PRA and SNS traffic. Although the native atria are left intact and the sensory afferents are not intentionally severed, several lines of evidence indicate that they may be reduced in number or permanently ablated. Impaired firing patterns of atrial mechanoreceptors have been observed during CHF when it is complicated by chronic atrial distension (Zehr et al., 1971; Greenberg et al., 1973). This is a pre-existing condition for most HTR. Additionally, the increased sympathetic activity present during heart failure has been reported to increase coronary vasoconstriction and induce myocardial tissue damage in certain types of CHF (Swedberg et al., 1979; Swedberg et al., 1990). These findings indicate that the native afferent receptor sites themselves may deteriorate or lose sensitivity. Finally, the native atria are permanently devascularized during Tx and must rely upon collateral circulation (Baumgartner et al., 1990). Thus, native atrial stretch receptor afferents may be impaired or permanently ablated as a consequence of

preTx CHF and/or as a result of surgical devascularization. The present study, however, was not designed to isolate cardiac stretch receptor reflexes. Exclusive tests of the afferent part of cardiac reflex arcs are difficult to devise in humans. Nonetheless, the inference drawn from our indirect data is that cardiac deafferentation potentiates the neuroendocrine response to exercise.

Hemodynamic Responses to Exercise

The hemodynamic data demonstrates that the transplanted denervated heart has the capacity to augment cardiac output early (within 30 seconds) after the onset of upright cycle exercise (figures 8 and 9). The relative increases in cardiac output ($\% \Delta$ from baseline) were similar for both groups during the initial 2 to 3 minutes of exercise at 40 and 70% of peak power output. These findings were surprising considering the attenuated chronotropic response of the denervated heart. Numerous other studies have reported cardiac output dynamics in HTR but the first available measurements of cardiac output in those investigations are several minutes after the commencement of exercise (Stinson et al., 1972; McLaughlin et al., 1978; Pope et al., 1980; Pflugfelder et al., 1987; Pflugfelder et al., 1988; Younis et al., 1990; Rudas et al., 1990). Banner et al. (1988) used Doppler ultrasound to measure relative changes in stroke volume and cardiac output every 30 seconds during the rest to exercise transition in HTR. They reported diminished stroke volume and cardiac output responses early (30 seconds and 1 minute) during upright exercise at the same absolute exercise intensity compared to the control group. Our HTR

demonstrated increased stroke volume but similar cardiac output responses ($\% \Delta$ from baseline) early after the onset of exercise when compared to control subjects. Our data, however, refer to exercise performed at the same relative intensity and are therefore not incompatible with the previous research.

The major difference between HTR and the control subjects was their inability to immediately increase heart rate at the onset of exercise. The transplanted heart increased rate in a gradual linear fashion throughout the entire 10 minutes of each submaximal exercise test. Contrastingly, heart rate in the control group increased dramatically at the initiation of exercise and reached steady-state levels by 2 minutes during exercise at 40% of peak power output. Both groups experienced a slow upward drift in heart rate during exercise at 70% of peak power output but the $\% \Delta$ from baseline during the first minute after the commencement of exercise was 10 fold greater in the control group.

Unlike the control group, which simultaneously increased both heart rate and stroke volume, the primary mechanism for augmenting cardiac output during exercise in HTR was increased stroke volume with little change in heart rate. Previous investigations using radionuclide angiography, flouroscopy and thermodilution techniques have shown that the increases in stroke volume during mild exercise in HTR are mediated by an increase in end-diastolic volume rather than a decrease in end-systolic volume (Stinson et al., 1972; McLaughlin et al., 1978; Schroeder et al., 1979; Pope et al., 1980; Pflugfelder et al., 1987; Younis et al., 1990; Rudas et al., 1990). Our hormonal data support those findings by demonstrating that moderate exercise (40% of peak power output) evoked

a neuroendocrine response (assuming plasma NE and PRA as markers) in both groups that was 2 to 3 fold greater than baseline. This level of sympathetic arousal may elevate circulating catecholamines to concentrations that begin to have chronotropic effects upon the heart but are still below the threshold concentration necessary for an inotropic effect (Clutter et al., 1980). Thus, catecholamine mediated increases in contractility would be expected to be slight. The observed increase in stroke volume occurs, therefore, as a result of increased preload (Frank-Starling mechanism) presented to the heart by the "muscle pump" action in working limbs and the "respiratory pump" action of increased ventilation.

At higher intensity exercise (70% and 100% of peak power output) SNS overactivity was observed in HTR with plasma NE reaching levels that were 7 fold greater than baseline. It is known that when plasma E concentrations exceed basal levels by 4 to 5 fold, E stimulates beta-adrenergic receptors in the heart causing increased contractility (Clutter et al., 1980; Pope et al., 1980; Port et al., 1990). Although we did not measure plasma E, it is reasonable to postulate from our NE data that plasma E concentrations had risen to the necessary levels to behave as a circulating beta-adrenergic agonist and compensate the denervated heart through increased rate and contractility. The full effect of E on the heart is observed because it concurrently increases splanchnic, skeletal muscle and total vascular conductance enough so that rising cardiac output is unimpeded by increased afterload and opposing baroreflexes.

In contrast to E, plasma NE levels above 1.8 ng/ml (4 to 5 times normal resting values) behave as a circulating alpha-adrenergic hormone

and cause vasoconstriction (Silverberg et al., 1978). The observed average plasma NE concentration for HTR at maximal exercise was 3.9 ng/ml (9 to 10 times higher than normal resting values) and potentially could have imposed limits on exercise capacity by increasing peripheral vascular resistance (afterload). The rise in ventricular afterload tends to reduce stroke volume in normal innervated subjects and oppose further increases in cardiac output through arterial baroreflex (Sagawa, 1983; Rowell, 1986). Thus, the magnitude of pressor hormones (NE, AVP and PRA) released in HTR during exercise may be potentially detrimental to exercise hemodynamics.

Pulmonary Function

Pre- vs PostTransplantation Pulmonary Function Tests

General pulmonary function was improved following Tx. Resting ABG values were improved but did not reach statistical significance from preTx. However, given the increases observed in resting PaO₂ and PaCO₂, the resting alveolar to arterial gradient for oxygen partial pressure improved from 12.6 before surgery to 3.3 mmHg after Tx. Pulmonary ventilatory function (FVC and FEV₁) improved 10-14% and DLCO also increased 6% in HTR from pre- to postTx. Others have reported diminished DLCO in HTR (Casan et al., 1987; Banner et al., 1988). Immunosuppression therapy with cyclosporine, the surgical procedure and reduced hemoglobin have been cited as possible mechanisms responsible for the diminished DLCO (Casan et al., 1987; Wright et al., 1990; Bussieres et al., 1990). However, the nature of the DLCO impairment

in HTR is unknown and generalizations should not be made because the available data does not include pre- and postTx pulmonary measures. Considering the fact that DLCO is markedly diminished in many CHF patients awaiting Tx (McNeil et al., 1958; Morley et al., 1989; Wright et al., 1990; Bussieres et al., 1990), it is possible that the abnormal postoperative DLCO observed in some HTR may not be a result of the surgical procedure or immunosuppression therapy but rather a persistence of pre-existing conditions.

Our finding of a 6% improvement in DLCO following surgery is not in agreement with the only previous study that has reported both pre- and postTx DLCO values in HTR (Casan et al., 1987). The data of Casan et al. (1987) showed a mean reduction of 14% in DLCO in 10 HTR 6 to 12 months after surgery despite significant improvements in FVC and FEV₁. The authors concluded that the reduction in DLCO was an early manifestation of cyclosporine induced lung toxicity similar to that which has been documented in the kidneys of renal transplant patients (Myers et al., 1984; Taube et al., 1985; Humes et al., 1985; Racusen et al., 1986; Macris et al., 1989). As indirect evidence for their conclusion, they reported a high correlation ($r=0.87$) between the reduction in DLCO and the level of cyclosporine in whole blood at the time of the pulmonary function tests. In the present study, however, the correlation coefficient for the relationship between the change in DLCO from pre- to postTx and whole blood cyclosporine levels was $r=0.08$ ($p=0.822$). DLCO either increased (5/10 patients) or remained approximately the same (5/10 patients) for the HTR tested before and after Tx in the present study.

One possible explanation for these divergent findings is that the cyclosporine whole blood levels attained in our HTR (mean value of 542 ng/ml) were on the low side of the therapeutic range (table 5). Casan and coworkers (1987) reported cyclosporine whole blood levels that were 70% higher (mean value of 923 ng/ml). Another point to consider, however, is that HTR in the present study were older (50 vs 39 years) and had also been on cyclosporine therapy longer (18 vs 9 months) at the time of the study. If cyclosporine immunosuppression is complicated by the development of lung toxicity, it seems reasonable that we would have observed more advanced manifestations of pulmonary dysfunction in the present study. However, correlational analysis indicated that a relationship did not exist between the change in DLCO from pre- to postTx and the age of HTR ($r=0.34$; $p=0.34$) or the number of months on cyclosporine therapy ($r=0.09$; $p=0.79$). Previous smoking history (pack years) was the only variable found to be significantly and inversely related to DLCO before ($r=0.80$; $p\leq 0.05$) and after Tx ($r=0.77$; $p\leq 0.05$).

Cyclosporine immunosuppression therapy is frequently complicated by the development of hypertension and early nephrotoxicity or hepatotoxicity (Myers et al., 1984; Taube et al., 1985; Humes et al., 1985; Racusen et al., 1986; Macris et al., 1989). Additionally, neurologic toxicity has been observed in liver transplant patients and HTR receiving cyclosporine (Berden et al., 1985; Adams et al., 1987; DeGroen et al., 1987; Vazquez de Prada et al., 1990). Several other side effects of cyclosporine have been reported as isolated case reports but none have been considered contraindications for cyclosporine therapy (Vazquez de Prada et al., 1990). Evidence for cyclosporine induced pulmonary dysfunction, however, has

not been presented in the related literature. Bennet and Norman (1986) in an earlier review make no reference to the adverse effects of cyclosporine on the lung. Copeland et al. (1986) describe only infrequent pleural effusion secondary to cyclosporine therapy and these symptoms are transient. In a detailed review of drugs potentially toxic to the lung, Cooper et al. (1986) do not include cyclosporine. Macris et al. (1989) in a recent review of clinical contraindications for cyclosporine immunosuppression therapy make no mention of pulmonary dysfunction.

Another non-pharmacologic consideration when comparing pre- and postTx pulmonary function in HTR is whether the patient was hospitalized during the wait for a suitable donor heart to become available. Patients who require continuous hospitalization before Tx are generally more critically ill than other transplant candidates (Macris et al., 1989). Such patients may be less likely to achieve normal pulmonary function preoperatively and have greater potential to improve DLCO following Tx. However, this notion was not supported by the results of the present study. Three HTR in the present study required hospitalization during their wait for donor organs (subjects 3, 5 and 11; table 5). Two of the hospitalized HTR achieved normal DLCO values ($DLCO > 70\%$ predicted) before Tx and did not show any change in DLCO from pre- to postTx. Unfortunately, preTx pulmonary function test values were not available for the third patient.

Pulmonary Diffusion Capacity and Exercise-Induced Hypoxemia

Although our data do not support the hypothesis that Tx and subsequent cyclosporine immunosuppression therapy adversely affect

DLCO, we did find resting diffusion abnormalities and arterial hypoxemia during strenuous exercise in five of eleven HTR. All of the control subjects maintained PaO_2 within 5 mmHg of their corresponding baseline values during exercise at 70% of peak power output and five HTR reduced PaO_2 5-10 mmHg below resting values. In the remaining five HTR, PaO_2 was reduced 15-38 mmHg below resting values to <90 mmHg in all cases, between 80 and 90 mmHg in two cases, between 70 and 80 mmHg in one case and to less than 70 mmHg in two cases (figure 4). The five HTR experiencing exercise PaO_2 below 90 mmHg were the only subjects in the study to have pulmonary DLCO that was $<70\%$ of predicted. The observed hypoxemia in HTR during strenuous exercise was not associated with a sluggish ventilatory response. Of the five HTR exhibiting exercise-induced hypoxemia, all showed substantial hyperventilation (PaCO_2 28-33 mmHg)(figure 5). These data imply that the reserves for gas exchange available to HTR with poor DLCO are insufficient to meet the metabolic requirements of strenuous exercise. It can reasonably be assumed that further reductions in PaO_2 occurred at maximal effort, although we did not measure ABG during the SL-GXT.

Our data do not provide an answer to the question of how much peak $\dot{\text{V}}\text{O}_2$ may have been reduced by DLCO impairment and consequent exercise-induced hypoxemia. There was not a significant difference in peak $\dot{\text{V}}\text{O}_2$ between HTR with normal and abnormal DLCO. However, the group with low DLCO was 8 years younger and, therefore, would typically be expected to have higher $\dot{\text{V}}\text{O}_{2\text{max}}$ values. Previous data suggest that an exercise-induced reduction in arterial oxygen saturation (SaO_2) to 92-93% is sufficient to cause a measurable effect on $\dot{\text{V}}\text{O}_{2\text{max}}$ and that, on the

average, this effect approximates about a 1% decrement in $\dot{V}O_{2\max}$ for each 1% decrease in %SaO₂ (Powers et al., 1989). However, %SaO₂ is determined not just by PaO₂ but by pH and temperature of arterial blood. During exercise at 70% of peak power output, two HTR with low DLCO achieved an exercise-induced metabolic acidosis of 7.28 arterial pH, a PaO₂ of 60-63 mmHg and an assumed vascular temperature increase of 1.5°C. These factors are sufficient to reach a desaturation of 86-88% SaO₂ and cause a 8-10% reduction in peak $\dot{V}O_2$. At maximal exercise, more severe metabolic acidosis (pH ~7.20) and hypoxemia (PaO₂ ~60 mmHg) could conceivably be attained by HTR in the present study. These changes may reduce SaO₂ to 80-85% and cause 10-15% reductions in peak $\dot{V}O_2$ (Powers et al., 1989).

Nonsignificant decreases of arterial PO₂ during peak exercise have been reported in heart (Degre et al., 1987) and combined heart-lung (Theodore et al., 1987) transplant recipients. However, pulmonary function data were not reported and it is possible that diffusion impairment was not present in those transplant recipient samples. We are unaware of other investigations that have measured ABG during exercise in HTR. Although our data show that pulmonary abnormalities are common and may be severe enough to limit exercise capacity, survival after Tx appears to be unrelated to the presence or extent of such abnormalities. Bussieres et al. (1990) evaluated the pulmonary status of 33 CHF patients who subsequently underwent Tx. Six of the 33 patients died within 9 months of Tx. Eight of the survivors would have failed previously published pulmonary test criteria for HTR because they had FVC, FEV₁ and DLCO <60% of predicted (Stevenson et al., 1986). In four

survivors DLCO was 50% of predicted, whereas no patients in the fatality group had a DLCO value that low.

Cardiac Denervation and Exercise Hyperpnea

Composition of ABG during exercise can be used as an index for determining adequate exercise hyperpnea. Our data demonstrate that, during moderate exercise (40% of peak power output), HTR with normal DLCO are capable of essentially normal pulmonary ventilation.

Numerous hypotheses involving neural, humoral and combined neural-humoral mechanisms have been postulated for control of exercise hyperpnea and support for each has been found (Powers and Beadle, 1985). Most investigators agree that the initial fast phase (phase 1) of exercise hyperpnea is neurally mediated. However, the site of origin and magnitude of contribution remain contentious. The fast phase has been demonstrated to be mediated indirectly through feedback via afferent stimuli originating from skeletal muscle in the working limbs (Kao, 1963; Tibes, 1977) or directly via feedforward by efferent signals from higher centers of the brain (Eldridge et al., 1981).

Another neurogenic hypothesis to explain the immediate hyperpnea observed at the onset of exercise contends that the ventilatory response is dependent on the cardiac output response. Afferent signals from cardiac ventricular stretch receptors have been shown to stimulate ventilation in animals (Wasserman et al., 1974, Uchida, 1976; Jones et al., 1982). Jones et al. (1982) found that the relationship between minute ventilation and changing right ventricular load in anesthetized dogs was $r=0.94$ ($p \leq 0.01$).

Cardiac deafferentation of the donor organ, a necessary consequence of Tx, would be expected to ablate reflex stimuli originating from the heart

thereby reducing the ability to appropriately control pulmonary ventilation. Deafferentation would be expected to result in hypoventilation during the onset of exercise and be characterized by transient hypercapnia and hypoxemia. Our ABG data show that absolute changes in PaO_2 , PaCO_2 and pH in HTR with normal DLCO were not different from control subjects during the transition from rest to exercise at 40% of peak power output. Although the results of the present study do not refute the possible existence of a cardiodynamic reflex, they may be interpreted as an indication that the afferent cardiac stimuli are not essential in the regulation of pulmonary ventilation during the onset of moderate exercise. The absence of hypercapnia in denervated HTR may be interpreted as further evidence of the existence of redundant mechanisms capable of stimulating ventilation.

Skeletal Muscle Strength

It has been suggested that skeletal muscle weakness may be a primary mechanism for exercise intolerance in otherwise healthy HTR (Massie et al., 1988; Kavanagh et al., 1988). Preoperative inactivity due to severely limited cardiac output and an extended waiting period for a donor organ to become available results in considerable deconditioning and muscle atrophy. Following Tx, chronic corticosteroid therapy (ie. prednisone) for immunosuppression is recommended for most HTR. Corticosteroids have been known to cause pronounced atrophy of skeletal muscle (steroid myopathy) when administered at high doses for prolonged periods of time (Goldberg and Goodman, 1969; Dujovne and Azarnoff, 1975; Kinney and Felix, 1979; Almon and Dubois, 1990). However, we are unaware of previous data describing the muscular strength of HTR.

Evidence of reduced muscle mass was not apparent in our HTR. Mean body weight was nearly identical in both groups (85.5 vs 85 kg) but HTR did have 2.2 kg less lean body mass than the control group. However, this difference was not statistically significant. Although immediate postTx prednisone dosage is high for HTR, the relatively low maintenance dose of prednisone taken by these patients (mean dosage=10mg/day) appears to be below the threshold level necessary to evoke significant catabolic activity and muscle wasting.

Leg strength was markedly reduced in HTR despite the fact that predicted lean body mass was approximately the same as the control group. Absolute 1-RM knee-extension strength in HTR was 69% of that achieved by the control group. A strength difference of this magnitude cannot be explained by the 2.2 kg difference in lean body mass between the two groups. When 1-RM was expressed in relative terms, the amount of weight lifted per unit of lean body mass by HTR was 72% of that achieved by the control group. Furthermore, the knee-extension leg strength of HTR does not compare favorably with data previously collected in our laboratory using the same Nautilus tensiometer (Braith et al., 1991). The 1-RM knee-extension strength of HTR was 75% of that achieved by a group of 58 untrained and sedentary subjects (n=33 men; n=25 women). Although HTR were older (50 vs 25 years of age), it must also be acknowledged that nearly one half of the subjects measured by Braith et al. (1991) were females, whereas 10 of the 11 HTR were male.

The explanation for this imbalance between lean body mass and muscular strength may be related to preTx deconditioning. Muscular strength can deteriorate through neural as well as morphological

mechanisms (Moritani and deVries, 1979; Moritani and deVries, 1980). Prolonged inactivity may reduce the number of motor units recruited to perform a specific task or result in inefficient motor unit recruitment patterns (Moritani and deVries, 1979) and decrease the period of time that motor units are active (Grimby, 1981). Additionally, Moritani and deVries (1980) have shown that neural factors play an increasingly important role in muscle strength in older individuals.

Abnormalities in skeletal muscle metabolism are common in CHF patients before Tx (Massie et al., 1988). The most prominent findings have been increased glycolytic metabolism at submaximal work rates but impaired oxidative phosphorylation (Massie et al., 1988). Although we did not find evidence of muscle tissue wasting in HTR, corticosteroid therapy may still have contributed to the observed reduction in strength. Prednisone is known to reduce muscle membrane excitability (Gruener and Stern, 1972). Gruener and Stern (1972) found that isolated muscle preparations from animals treated with corticosteroids required a more hyperpolarized membrane potential in order to be able to generate an action potential. The authors concluded that prednisone appears to adversely affect membrane permeability or cause a reduction in intracellular ions. Thus, intrinsic changes in skeletal muscle energetics related to CHF before Tx and prednisone induced changes in recruitment patterns or contractile efficiency after Tx may cause strength decrements without significant muscle tissue wasting.

Summary and Conclusions

The ability of the transplanted human heart to function during physical exercise has a direct bearing on the usefulness of Tx. Previous investigations have studied the traditional importance attributed to hemodynamics of the denervated heart. We have demonstrated in a group of eleven HTR that arterial hypoxemia, SNS overactivity and skeletal muscle strength deficiencies may also contribute significantly to the limitation of exercise capacity.

Pulmonary Gas Exchange Abnormalities

Although we have shown that Tx and subsequent immunosuppression does not have an adverse effect upon DLCO, postTx pulmonary function appears to impose limitations on exercise capacity, specifically when DLCO is less than 70% of predicted. Five of the eleven HTR studied experienced hypoxemia (PaO_2 15-38 mmHg below resting values) during submaximal exercise and all five presented with resting DLCO values <70% of predicted. It should be assumed that further substantial reductions in PaO_2 occur at maximal effort and are detrimental to $\text{VO}_{2\text{max}}$.

Sympathetic Nervous System Overactivity

A major contribution of this study is the finding that the neuroendocrine response to exercise performed at the same relative intensity is greater in HTR compared to innervated control subjects. A threshold exercise intensity of >40% of peak power output is required to evoke SNS overactivity. We interpret our findings to be the result of cardiac deafferentation and consequent unopposed vasomotor stimulation.

The clinical implications of these findings are currently speculative but may offer a rationale for the early introduction of therapy designed to buffer SNS overactivity (eg. beta-adrenergic blockade and demand pacemaker). Although circulating catecholamines provide chronotropic and a measure of inotropic support for the denervated heart, extreme SNS arousal increases peripheral vascular resistance (afterload) and the work of the heart while opposing increases in stroke volume. Beta-blockade buffers SNS overactivity and improves survival in diverse cardiovascular disease populations, suggesting that chronic SNS stimulation plays an important role in cardiovascular pathology.

Skeletal Muscle Strength Deficiencies

The most popular instruments in use today for measurement of an individual's exercise capacity are the stationary cycle and treadmill. It is assumed that muscular fatigue will not significantly limit cardiovascular performance. Our data suggest, however, that skeletal muscle weakness may be a primary mechanism for reduced exercise capacity in otherwise healthy HTR. Knee-extension strength (quadriceps) in HTR was 69% of that achieved by the control group, despite the fact that both groups were identical in body weight and similar in lean body mass. Thus, it is not surprising that ride time to exhaustion, peak power output (watts) and $\dot{V}O_2\text{max}$ were all approximately 60% of the values attained by the control group. These data demonstrate that early progressive resistance training is indicated for HTR to reverse the strength deterioration associated with preTx CHF and to protect against possible postTx steroid myopathy.

APPENDIX

PROTOCOL NUMBER 59-90

INFORMED CONSENT TO PARTICIPATE IN RESEARCH

J. HILLIS MILLER HEALTH CENTER

UNIVERSITY OF FLORIDA

GAINESVILLE, FLORIDA 32610

You are being asked to volunteer as a participant in a research study. This form is designed to provide you with information about this study and to answer any of your questions.

1. TITLE OF RESEARCH STUDY

Select cardiovascular and respiratory responses to exercise in the heart transplant recipient.

2. PROJECT DIRECTOR

Name: Michael L. Pollock, Ph.D., E. D. Staples, M.D.; William Mayfield, M.D.
Telephone Number: (904)392-9575

3. THE PURPOSE OF THE RESEARCH

The purpose of the study is to determine those mechanisms which may limit physical work tolerance in men and women who have undergone orthotopic heart transplantation.

4. PROCEDURES FOR THIS RESEARCH

Visit 1: During your first visit to the Center for Exercise Science you will fill out a medical history form and undergo an examination by a physician. Your body fat and muscle mass will be assessed by measuring skinfold fat thicknesses and body circumferences. A small plastic line will be placed in a vein in your arm so that a one teaspoon blood sample can be drawn before and after a stationary cycle exercise capacity test (for a total of two teaspoons). Your ECG and cardiac output (blood pumped by heart) will be monitored during the test from adhesive electrodes placed on your chest. You will have a rubber mouthpiece in your mouth and a clip on your nose so that your expired air can be collected. The exercise test will start out very light and every three minutes the exercise will become somewhat more difficult until the physician stops the test because of certain signs or symptoms, or when you are unable to continue because of fatigue. If you have no cardiovascular or other significant medical abnormalities you will be included in the study. (Total time for visit one will be 2 hours)

Visit 2: Visit two will also be at the Center for Exercise Science. You will first report to the Pulmonary Laboratory to have your pulmonary function test. You will be asked to inhale maximally and then exhale into a spirometer. You will be seated throughout the test.

You will then be taken to the Metabolic Testing Laboratory and be prepared for a stationary bike ride while your heart and lung function are measured. A supervising physician will be present to insert a small plastic line in an artery in your arm so that arterial blood samples may be drawn before and during exercise to measure the amount of oxygen and blood pressure hormones in your blood. Your ECG and cardiac output will also be monitored before and during exercise from adhesive electrodes placed on your chest.

After the catheter has been inserted and baseline resting measurements are completed, you will perform two bouts of stationary cycling exercise with a rest period between. The first exercise period is performed at 40% of your capacity (light exercise) and will last approximately 6 minutes. The second exercise period is performed at 60% of your capacity (moderate exercise) and will last approximately 9 minutes. A total of approximately 40-50 ml (3 tablespoons) of blood will be drawn during each exercise bout. (The total time of your second visit will be 3 1/2 hours)

5. POTENTIAL RISKS OR DISCOMFORTS

Certain risks exist when testing any individual during exercise that includes intensities up to and including a maximal effort. This risk is about 1 non-fatal incident (event) in approximately 5,000 maximal treadmill tests. The Cooper Clinic in Dallas, Texas has had 6 non-fatal and no fatal cardiovascular events in 80,000 exercise tests. Exercise testing in cardiac disease patients results in about 1 fatal event per 20,000 tests. The principal investigators are experienced with exercise testing in cardiac patients and normal controls. All subjects will have undergone a resting ECG, physician's physical examination, and cardiovascular examination before the exercise test; the heart rate, blood pressure, and ECG responses will be monitored closely during the tests. In addition, the tests will be supervised by a physician experienced in graded exercise testing.

There is a minimal risk associated with blood drawing and catheter placement. The risks of drawing blood from a vein include discomfort at the site of injection; possible bruising and swelling around the injection site; rarely an infection; and, uncommonly, faintness from the procedure. The investigators involved in these studies have used these techniques for 12 or more years and have drawn over 5,000 blood samples in that time.

6. POTENTIAL BENEFIT TO YOU OR TO OTHERS

You will receive a complete cardiovascular and pulmonary exam as well as body fat measurement and a battery of other tests during the course of the study that would otherwise be very costly. The results of these tests will be available to your private physician. The major benefit is to the medical community and specifically the heart transplant recipient. You will have helped to further our understanding of the mechanisms which may diminish exercise tolerance in transplant patients. This knowledge may then be applied toward improving the likelihood of a heart transplant recipient returning to work and leisure time activities.

7. ALTERNATIVE TREATMENT OR PROCEDURES, IF APPLICABLE

The only alternative available to you is to not enroll or withdraw from the study. A decision by transplant patients to not enroll or withdraw from the study at any time will not influence the continued post-operative care provided by the transplantation team.

8. GENERAL CONDITIONS

I understand that I will ____ / will not XX receive money for my participation in this study. If I am compensated, I will receive

I understand that I will ____ / will not XX be charged additional expenses for my participation in this study. If I am charged additional expenses these will consist of _____

I understand that I am free to withdraw my/my child's consent and discontinue participation in this research project at any time without this decision affecting my/my child's medical care. If you have any questions regarding your rights as a subject, you may phone 392-3063.

In the event of my/my child's sustaining a physical injury which is proximately caused by this experiment, Professional Medical care received at the J. Hillis Miller Health Center exclusive of hospital expenses will be provided me without charge. This exclusion hospital expenses does not apply to patients at the Veterans Administration Medical Center (VAMC) who sustain physical injury during participation in VAMC- approved studies. It is understood that no form of compensation exists other than those described above.

I also understand that the University of Florida and the Veterans Administration Medical Center will protect the confidentiality of my records to the extent provided by Law. The Study Sponsor, Food and Drug Administration or either Institutional Review Board may ask to review my records, however, the records will remain confidential as only a number and initial will be used.

9. SIGNATURES

I have fully explained to _____
the nature and purpose of the above-described procedure and the benefits
and risks that are involved in its performance. I have answered and will
answer all questions to the best of my ability. I may be contacted at
telephone number _____

Signature of Principal or Co-Principal Investigator Obtaining Consent	Date
--	------

I have been fully informed of the above-described procedure with its
possible benefits and risks and I have received a copy of this description. I
have given permission of my/ my child's participation in this study.

Signature of Patient or Subject or Relative or Parent or Guardian (specify)	Date
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Signature of Child (7 to 17 yrs. of age)	Date
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Signature of Witness	Date
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BIOGRAPHICAL SKETCH

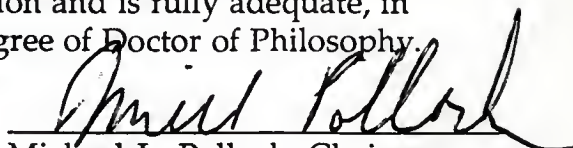
Randy Braith was born in Staples, Minnesota on January 15, 1950. He was graduated from Staples High School in June, 1968.

In May 1973, he completed a Bachelor of Science degree at Minnesota's Bemidji St. University with a major in English. He taught high school English before entering graduate school at Minnesota's St. Cloud State University where he completed the Master of Science degree in exercise physiology in February, 1983.

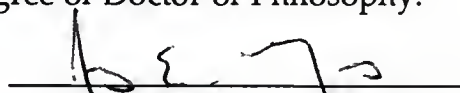
In March 1983, he accepted a position as Director of Pulmonary Rehabilitation at Medical Center Hospital at Punta Gorda, Florida and also managed two hospital-based Wellness Centers in Charlotte County, Florida. In September 1986, he became Laboratory Coordinator for the newly created Center for Exercise Science at the University of Florida.

He entered graduate school at the University of Florida in August 1987 to pursue the Doctor of Philosophy degree with a major in exercise and sport sciences and a minor in physiology. His degree program will be completed in 1991. Following graduation, he will pursue an academic career.

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Michael L. Pollock, Chairman
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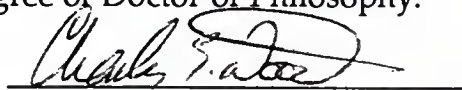
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Associate Professor of Exercise
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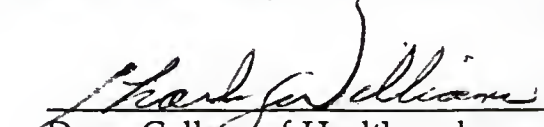

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This dissertation was submitted to the Graduate Faculty of the College of Health and Human Performance and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1991



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